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TITLE OF THESIS/*TITRE DE LA THÈSE* Utilization of Energy Substrates by Ruminants Exposed to Cold

UNIVERSITY/*UNIVERSITÉ* University of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED/*GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* Ph.D.

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE DEGRÉ* 1974

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THE UNIVERSITY OF ALBERTA

UTILIZATION OF ENERGY SUBSTRATES BY RUMINANTS EXPOSED TO COLD

BY

DONALD GORDON McKAY

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY
IN
ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Utilization of Energy Substrates by Ruminants Exposed to Cold submitted by Donald Gordon McKay in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Physiology.

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ABSTRACT

The utilization of carbohydrate and fat by ruminants for cold induced heat production was studied in closely shorn ewes. Three sheep were "warm conditioned" in a chamber maintained at 18 ±2°C and another three were "cold conditioned" in a chamber maintained at -2 ±2°C for a minimum of 4 weeks prior to any trials. Isotopically labelled glucose or palmitate was continuously infused into the sheep during each trial in which sheep were exposed to 18 ±2, -2 ±2 or -24 ±2°C in either a fed or fasted state. The recovery of labelled palmitate from plasma was not satisfactory. Therefore parameters of palmitate metabolism were estimated from the plasma palmitate concentrations and the specific activities of the CO_2 produced.

In most cases the hematocrits and heat production of sheep exposed to cold (-2 or -24°C) were higher and the respiratory quotients lower than those of sheep exposed to warm (18°C).

Sheep chronically exposed to 18°C had an average glucose flux of 1.24 mg/min/kg when fed and 1.13 mg/min/kg when fasted. Approximately 19% of the glucose fluxes in these sheep were oxidized to CO_2 which accounted for approximately 3.5% of the total heat production in both fed and fasted states. Palmitate accounted for a similar percent of the heat production as glucose when the sheep were in a fed

state, however the estimates of the percent heat production from palmitate were 2-3 times greater when the sheep were in a fasted state. Fasting generally increased the percent of the heat production derived from palmitate in both warm and cold conditioned sheep and decreased the percent of the heat production derived from glucose in cold conditioned sheep. The percent of the heat production derived from glucose and palmitate in fed sheep chronically exposed to -2°C was approximately 125% and 28% greater, respectively, than those observed for fed sheep chronically exposed to 18°C. The percent of the heat production derived from glucose and palmitate in fed warm conditioned sheep acutely exposed to -24°C was approximately 125% and 200% greater, respectively, than those observed for the warm conditioned sheep chronically exposed to 18°C. The cold conditioned sheep had higher rates of glucose oxidation and slightly lower rates of palmitate oxidation than warm conditioned sheep at either 18 or -24°C when fed. However, the percent of the glucose flux oxidized to CO_2 in the cold conditioned sheep was less than that of the warm conditioned sheep when fed and became even less when fasted.

It is concluded that carbohydrate as well as fat are utilized for cold induced heat production in ruminants. Further, cold conditioning enhances the role of glucose in heat production in fed sheep.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. L.W. McElroy, past Chairman of the Department of Animal Science and Dr. L.P. Milligan, present Chairman of the Department of Animal Science for the use of the Department's facilities.

Sincere thanks are extended to my supervisor, Dr. B.A. Young, Associate Professor of Animal Physiology. The author also expresses gratitude to Dr. J.R. Thompson and Dr. L.P. Milligan for helpful suggestions, criticisms and guidance during the course of this work and to Mr. R. Weingardt for his help in computing the data.

Appreciation is extended to Mr. B. Turner, Mr. T. Fenton, Mr. B. Kerrigan and Mr. N. Arborn for their technical assistance.

The author also wishes to thank Mrs. C. Preston, who undertook the task of typing this manuscript.

The author is grateful to his wife, Maureen, for her understanding and encouragement throughout this study.

Financial assistance for the study was received from the National Research Council of Canada and the University of Alberta in the form of a Dissertation Scholarship. These are gratefully acknowledged.

TABLE OF CONTENTS

	Page
Introduction	1
Literature Review	3
A. Substrates Used for Heat Production	5
a) Energy substrates from body	5
b) Energy substrates from diet	7
c) Glucose requirements of ruminants	9
d) Sources of glucose for ruminants	11
e) Possible glucose sparing mechanisms in ruminants	14
B. Availability of Substrates	16
Nutritional status and substrate availability	16
C. Substrate Utilization for Cold Induced Heat Production	18
Materials and Methods	23
A. Experimental Animals	23
B. Experimental Procedures	24
C. Experimental Design	28
D. Infusates	30
E. Blood Sampling	30
F. Analytical Procedures	31
a) Respiratory gas analysis	31
b) Glucose concentration	31
c) Specific activity of glucose	31
d) Separation of free fatty acids	32
e) Specific activity of palmitate	32
G. Calculations	33
a) Respiratory quotient	33
b) Heat production	33
c) Specific activity	33

Page

Results 34

A. General Observations 34

B. Hematocrits 41

C. Respiratory Quotients 43

D. Heat Productions 45

E. Concentrations of Plasma Glucose and Palmitate 47

a) Concentrations of plasma glucose 47
b) Concentrations of plasma palmitate 50

F. Specific Activities of Plasma Glucose 51

G. Specific Activities of Plasma Palmitate 54

H. Specific Activities of CO_2 from Glucose and Palmitate 54

a) Specific activities of CO_2 from glucose 54
b) Specific activities of CO_2 from palmitate .. 57

Derived Results 59

A. Calculations of Glucose Parameters 59

B. Calculations of Palmitate Parameters 62

C. Summary of Derived Results 67

a) Glucose fluxes 67
b) Palmitate fluxes 70
c) Glucose oxidation rates 70
d) Palmitate oxidation rates 73
e) Percent glucose oxidized to CO_2 74
f) Percent palmitate oxidized to CO_2 74
g) Percent CO_2 from glucose 76
h) Percent CO_2 from palmitate 78
i) Percent heat production from glucose 79
j) Percent heat production from palmitate 82

Summary of Results 83

General Discussion 88

Summary and Conclusions 108

Bibliography 111

Appendix A Feed Test Report 121

Appendix B Methods 122

Appendix C Measurements by Trial 128

Appendix D Composition of Plasma Free Fatty Acids ... 162

LIST OF TABLES

Table		Page
1.	Possible Substrates for Glucose Synthesis in Sheep (Leng 1970)	14
2.	Experimental Design	29
3.	Hematocrits (%) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	42
4.	Respiratory Quotients of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18,-2 and -24°C.	44
5.	Heat Productions(cal/min/kg) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	46
6.	Concentrations of Plasma Glucose (mg/100ml) and Palmitate (umole/ml) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	48
7.	Standardized Specific Activities of Plasma Glucose of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	52
8.	Standardized Specific Activities of CO_2 Produced ($ucX10^2$/mg C) by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C..	55
9.	Fluxes of Plasma Glucose (mg/min/kg) and Palmitate (mmole/min/kg) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	68
10.	Oxidation Rates of Plasma Glucose (mg/min/kg) and Palmitate (mmole/min/kg) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	71
11.	Percent of the Glucose and Palmitate Oxidized to Carbon Dioxide by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	75
12.	Percent of the CO_2 Produced from Glucose and Palmitate by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C..	77

Table		Page
13.	Percent of the Heat Production Produced from Glucose and Palmitate by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C.	80
14.	Summary of Comparisons I, II and III of the Results	84
15.	Summary of Comparisons IV and V of the Results	85

LIST OF FIGURES

Figure		Page
1.	Arrangement of Sheep and Apparatus During a Trial	25
2.	Sequence of Events in Trials	27
3.	Results of a Typical Trial	37
4.	Results of a Typical Trial (cont'd)	38
5.	The Percent of the Increased Heat Production (Hp) Associated with Cold Exposure of Sheep Accounted for by Increases in Glucose and Free Fatty Acid (FFA) Oxidation	103

INTRODUCTION

Large numbers of ruminants exist in naturally cold regions such as Western Canada. Livestock producers in these regions must be aware of the effect of cold environments on animal production if they are to efficiently feed and maintain their animals. An understanding of animal homeothermy will better equip us to evaluate the effects of cold on animal production.

The contribution of the major substrates - carbohydrate, fat and protein - to the increased heat production associated with cold exposure in ruminants is in doubt. On the basis of respiratory quotients (RQ) Graham et al (1959) and Blaxter and Wainman (1961) suggested that sheep and cattle, respectively, utilized fat as the sole source of energy for the increased heat production. Pagé and Chénier (1953) made a similar suggestion for rats on the basis of RQ measurements. However, in 1966, Masoro, after reviewing the available literature concluded that although fat is a major fuel, all three energy sources - fat, carbohydrate and protein - are used for cold induced thermogenesis. His conclusion was based mainly on measurements of nitrogen excretion in rats and studies on rats where isotopically labelled glucose and palmitate were used.

In ruminants the majority of ingested carbohydrate

is converted in the rumen to volatile fatty acids (Eldsen and Phillipson 1948; and Heald 1952) thus limiting the amount of carbohydrate available for absorption. Ruminants, unlike monogastrics, must therefore rely largely upon endogenous formation of glucose (a major animal carbohydrate) to meet their metabolic requirements for glucose. Thus, there is still some doubt as to the availability and utilization of glucose by ruminants for the increased heat production associated with cold exposure.

The present thesis was undertaken to determine whether ruminants utilize glucose as well as fat for cold induced thermogenesis. Isotopically labelled glucose and palmitate were used to monitor glucose and palmitate metabolism in sheep acutely or chronically exposed to warm or cold temperatures.

LITERATURE REVIEW

Animals which regulate heat production and heat loss to maintain body temperature are called homeotherms. Most mammals and birds fall into this group (Brengelmann and Brown 1965). Homeotherms must decrease heat loss, increase heat production or both to maintain body temperature when exposed to cold. Heat loss is primarily regulated by physical means whereas heat production is regulated by chemical means. Examples of the physical means whereby an animal can regulate heat loss are: piloerection, postural changes and altering the blood flow to the periphery. Physical mechanisms of conserving heat are limited in the temperature changes with which they can cope. When temperatures fall below this limit additional heat is produced by oxidative processes (Evans 1956; and Carlson 1963). That temperature below which heat production in an animal increases is referred to as the animal's lower critical temperature (Blaxter 1958). Heat production in cattle (Blaxter and Wainman 1961) and sheep (Graham et al 1959) have been increased by exposing the animals to controlled cold environments. Webster (1966) and Webster et al (1969) reported that under severe cold stress the heat production of adult sheep may be increased by as much as five times the fasting heat production.

The capacity of an animal to increase its rate of heat production limits its resistance to severe cold.

It has been shown that an animal's resistance to cold is influenced by its previous exposure to cold temperatures (Hart 1957; Héroux 1963; Masoro 1966; Webster et al 1969; Slee 1972; Young 1972; and Young and Christopherson 1974). Slee (1972) found that the resistance of sheep to body cooling (measured during acute cold exposures) was generally increased by both previous chronic and acute cold exposures. The resting heat production and the capacity to increase this heat production during severe cold stress were observed by Webster et al (1969) to be enhanced by previous exposure of sheep to controlled cold temperatures. Although this work may be confounded by the increased food intake of the sheep when exposed to cold, recent results of studies on cattle fed at a constant level were published by Young in 1972 and Young and Christopherson 1974 which indicate that there is increased heat production in ruminants adapted to cold even when there is no direct cold stress imposed.

The increased heat production associated with cold may utilize energy substrates which would otherwise be available for production. The purpose of this review of literature is to attempt to answer the following questions:

1. What substrates can be used by an animal for heat production?
2. What factors affect the availability of the substrates?

3. What is currently known about substrate utilization in the ruminant for cold induced heat production?

A. Substrates Used for Heat Production

In this section we will be attempting to answer the question: What substrates can be used by an animal for heat production? There are only two sources of energy substrates normally available to an animal. One source is the animal's own body and the other source is the animal's diet.

a) Energy substrates from body

The body composition of a mature ruminant is approximately as follows (Crampton and Lloyd 1959):

Water	41 - 57%
Fat	18 - 41%
Protein	15 - 17%
Ash	3 - 4%
Carbohydrate	<1%

Only fat, protein and carbohydrate can be used by the animal as energy substrates.

Fat is usually the most abundant energy substrate and is distributed throughout the body in the adipose tissues. In terms of protection from cold, fat plays two roles; i. it can act as a source of energy for heat

production and ii. when distributed subcutaneously it increases thermal insulation thus reducing the requirement for greater heat production.

Protein, a major component of muscle is also relatively abundant in the animal body. However, the maintenance of protein mass is crucial to an animal's survival (Cahill et al 1970). Amino acids, the components of protein, can be oxidized directly or may first be converted to glucose via gluconeogenesis and then oxidized (Ford and Reilly 1969). The direct measurement of protein or amino acid utilization was not attempted in the present study.

Carbohydrate is found in the body chiefly in the form of liver glycogen, together with small amounts of muscle glycogen and blood sugar (Crampton and Lloyd 1959). Since carbohydrate is less than 1% of the total body composition it is evident that the amount of glycogen is limited (Cahill et al 1970; and Milligan 1971). The glycogen, when broken down into smaller compounds, yields glucose which can serve as an emergency fuel for severe stress or activity (Cahill et al 1970). Pernod et al (1972) suggested that the amount of liver glycogen in dogs may be a limiting factor during acute cold exposure because of the large hepatic glucose output. During prolonged cold exposure, the availability of carbohydrate depends on the

mobilization of glucose from glycogen, the rate of carbohydrate absorption from the gut and the rate of gluconeogenesis.

b) Energy substrates from diet

Fat, protein and carbohydrate are the major energy substrates present in the diet.

Fat usually makes up a small portion (<3%) of the ruminant's diet. However, short chain volatile fatty acids are formed from carbohydrate in the rumen and form a significant part of the absorbed energy. Seely et al (1967) suggested that the volatile fatty acids (VFA's) supply 60 - 80% of the daily metabolizable energy intake of sheep. Acetate, propionate and butyrate are the principle VFA's arising from fermentation in the rumen. A 50 kg sheep receiving 800 g of pelleted alfalfa hay daily absorbs between 100 and 250 g of acetate, 30 and 60 g of propionate and 5 and 15 g of butyrate per day (Ford 1965; Bergman and Wolff 1971). Bergman and Wolff (1971) found that the turnover of acetate by the liver in sheep accounted for only 4 to 14% of its net turnover and hence acetate must be utilized extensively by other tissue. Little or no butyrate appears in the portal blood (Annison et al 1957; and Bergman and Wolff 1971) since the majority of the butyrate is either converted to the ketone B-hydroxy-butyrate by the rumen epithelium (Leng and West 1969) or is

taken up by the liver (Bergman and Wolff 1971). In vitro studies support the theory that rumen epithelium converts substantial quantities of the butyrate to B-hydroxybutyrate (Pennington 1952; and Hird and Weidemann 1964). Propionate may be utilized to a small extent by the rumen epithelium and is otherwise largely removed by the liver (Leng and Annison 1963; and Bergman and Wolff 1971) with the result that levels of propionate in post-hepatic circulation are negligible.

The recommended crude protein content of a ruminant ration is 5 to 15% on a dry matter basis (NRC, 1970). Approximately 75% of the ingested nitrogen in the crude protein fraction would be absorbed in the form of amino acids. Amino acids can be used directly as energy substrates. Ford and Reilly (1969) found that 55 and 68% of the free amino acids were oxidized directly to CO_2 in sheep fed hay containing 49 g crude protein per day or a grass nut diet containing 88 g crude protein per day, respectively.

The largest portion of the ruminant's diet (80-85%) is carbohydrate. However, bacterial fermentation in the rumen reduces the majority of ingested carbohydrate to VFA's (Eldsen and Phillipson 1948; and Heald 1952) and hence only a small portion of the carbohydrate ingested is absorbed as glucose (Heald 1951; and Lindsay 1961). The small difference in glucose concentration observed between portal and carotid blood in sheep (Schambye 1951;

and Annison et al 1957) indicates a low rate of glucose absorption from the gut. On the basis of measurements of the net rate of glucose absorption via the portal blood in sheep, Bergman et al (1970) concluded that glucose absorption from the digestive tract did not occur in sufficient amounts to significantly augment total glucose availability. The amount of carbohydrate from roughage diets escaping fermentation to volatite fatty acids is small, although the amount from concentrate diets may be appreciable (Lindsay 1970). However Bergman et al (1970) found that more glucose was utilized by the portal drained viscera of sheep than was absorbed regardless of the diet which indicates that the requirements of sheep for glucose are greater than that supplied by the diet. Therefore ruminants must rely on endogenous synthesis of glucose to meet their daily requirements (Ford 1965; and Ballard et al 1969). What then are the requirements for glucose in a ruminant? This is an important question since an increase in glucose demand imposed by cold must also be met by endogenous synthesis.

c) Glucose requirements of ruminants

Acetate, the principle VFA absorbed from the gastrointestinal tract is a major energy substrate (Annison and Lindsay 1961) whose role as a fuel has been compared by Lindsay (1961) to that of glucose in the non-ruminant.

Acetate may be oxidized directly for energy or may be incorporated into fat stores for later use. Although acetate in the ruminant may be analogous in some ways to glucose in the non-ruminant, it can not meet all the demands for glucose in the ruminant. For example McClymont and Setchell (1956) were unable to demonstrate any uptake of acetate by sheep's brain in vivo, however, glucose uptake was substantial and its complete oxidation could account for the whole of oxygen consumption of this tissue. Significant amounts of glucose are utilized by neural, fetal and mammary tissue and portal drained viscera of ruminants (Bergman et al 1970). Glucose is required for the carbohydrate moiety of glycolipids, nucleic acids, mucopolysaccharides and for the glycerol of glycerides (Ford 1965).

Glucose also appears to play an important role in the metabolism of lipids. The oxidation of glucose via the pentose phosphate pathway is an important source of reduced nicotinamide adeninedinucleotide required for fatty acid synthesis (Ford 1965 and Ballard et al 1969). In vitro experiments in rat adipose tissue indicate that glucose and gluconeogenic substrates enhance lipolysis (Masoro and Panagos 1956; Ho 1970; and Ho et al 1970). Jarrett and Potter (1957) suggested that carbohydrate was required for the formation of oxaloacetate and that the concentration of oxaloacetate is a limiting factor in the

oxidation of acetate through the tricarboxylic acid cycle. If oxaloacetate is a limiting factor for the oxidation of acetate it would also be a limiting factor for the oxidation of longer chain free fatty acids.

Thus there is an apparent requirement for glucose in the ruminant as in the monogastric. This is reflected in the fact that there is little difference in the rate of glucose utilization between ruminants and monogastrics when the rates are expressed in terms of surface area (Baxter et al 1955) or body weight (Annison and White 1961; Kronfeld and Simesen 1961; Ford 1963; and Ballard et al 1969). Since very little glucose is absorbed in ruminants, these requirements must be met by the conversion of other substrates within the animal to glucose.

d) Sources of glucose for ruminants

Of the three major VFA's - acetate, propionate and butyrate - only propionate makes a net contribution to glucose synthesis (Black et al 1961; Annison et al 1963; Bergman 1963; and Annison and Linzell 1964) although all three may stimulate gluconeogenesis (Leng and Annison 1963; and Ballard et al 1969). The stimulation of gluconeogenesis by the VFA's is believed to arise from the activation of pyruvate carboxylase by short chain acyl coenzyme A molecules formed in the liver from the VFA's (Ballard et al 1969). The activation of pyruvate carboxylase increases the rate of formation of oxaloacetate, an important

substrate for gluconeogenesis.

On the basis of published results, Ford (1965) estimated that dietary propionate and absorbed glucose could supply only 33 to 67% of the total glucose requirement. In contrast, Leng et al (1967) found that enough propionate was produced in the rumen to account for 100% of the glucose requirements, however, upon measurement of propionate conversion to glucose, they found that only 54% of the glucose was derived directly from propionate. However, Leng's estimates account for only the direct conversion of propionate carbon to glucose carbon. It is probable that during the catabolism of propionate there are exchanges of carbon and hence propionate may contribute more carbon to gluconeogenesis indirectly. For example, if labelled propionate is metabolized to oxaloacetate (OAA) and enters the unlabelled OAA pool it is quite likely that unlabelled OAA may in turn be used for gluconeogenesis.

Two minor VFA's, valeric and isobutyric, are potential precursors for glucose synthesis however, they make up only 2-4% of the total VFA's (Leng 1970).

As well as glucose from VFA's some may be derived from amino acids. By the infusion of a mixture of ^{14}C-amino acids into sheep and comparing specific activities of plasma amino acids and glucose, Ford and Reilly (1969) estimated that between 11-17% of the glucose was derived

from amino acids. However, as pointed out by Lindsay (1970), the validity of such studies is questionable since it is not known to what extent plasma amino acids equilibrate with tissue amino acids or whether the equilibration is similar for all amino acids. There are also large differences in the ability of amino acids to contribute to glucose synthesis. Wolff et al (1972) estimated that amino acids contribute less than 25% of the glucose.

In times of active fat mobilization such as occur in undernutrition or starvation, the glycerol released from adipose tissue contributes significantly to glucose synthesis (Bergman 1968).

Table 1 from Leng's review (1970) of "Glucose Synthesis in Ruminants" summarizes the possible sources of glucose in a 35 kg sheep receiving 800 g of alfalfa chaff per day.

Propionate and amino acids have the greatest potential for glucose synthesis in fed sheep and may account for 40-70% of the glucose turnover (Bergman 1973). Glycerol largely replaces propionate as a glucose precursor in the fasted animal. Amino acids in the fasted animal account for approximately 70% of the glucose requirements (Bergman 1973).

Table 1. Possible Substrates for Glucose Synthesis in Sheep (Leng 1970)

Source of Glucose	Potential for glucose synthesis (g/day)	(mg/min/kg)
Ruminal propionate	85-90	1.69-1.79
Absorbed glucose	5	0.10
From glycerol of fat origin	6	0.12
Ruminal valeric & isobutyric acids	19	0.39
Amino acids from digestive tract	55-65	1.08-1.28
Total	170-185	3.38-3.68
Irreversible loss of glucose	86	1.71

Since the glucose requirement of ruminants must be met by other body substrates it is possible that glucose might be spared during times of increased energy demand such as that seen with cold.

e) Possible glucose sparing mechanisms in ruminants

Fasting may cause ketone production to increase in both ruminants (Palmquist 1972; and Leng and West 1969) and monogastrics (Masoro 1966; and Cahill et al 1970). During prolonged fasts in humans, the ketones replace a major share of the brain's glucose requirements (Owen et al 1967). Ketones are formed by the incomplete oxidation of fat and therefore the filling of glucose requirements with ketones will spare glucose and hence protein at the expense

of fat. A similar mechanism may exist in ruminants.

The recycling of glucose carbon via the Cori cycle might also be an important means of sparing glucose when gluconeogenic substrates are low (Leng 1970). It is also possible that glucose products might be used as catalysts to increase citric acid cycle intermediates, so that more FFA could be oxidized which in turn would spare glucose.

Unlike the monogastric, the ruminant converts very little glucose to FFA, and both the liver and adipose tissue have low levels of the key enzymes of the citrate cleavage pathway (Ballard et al 1969). Similarly glucose does not yeild FFA in the mammary tissue of ruminants (Lindsay 1971). Thus glucose is spared in the ruminant by the low conversion rate to FFA.

Glucose and protein may be spared by the action of growth hormone in times of increased energy demand. The concentration of growth hormone in the plasma is increased with stress (cold, pain, surgical stress, apprehension, etc.) fasting and exercise (Grodsky 1971). Olsen and Trenkle (1973) found increased levels of growth hormone in cattle exposed to controlled subzero temperatures. An excess growth hormone has been implicated in the etiology of ketosis (Seekles 1948; and Kronfeld 1965) and pregnancy toxemia (Reid 1968) in ruminants. It is well known that growth hormone impairs

glucose utilization (Hollobaugh et al 1968; Astwood 1970; and Grodsky 1971) and stimulates fat mobilization (Knobil and Hotchkiss 1964; Astwood 1970; and Grodsky 1971). Hence, there are feasible mechanisms which may act to spare glucose in the ruminant, however their role in cold thermogenesis is not well understood.

B. Availability of Substrates

Many factors affect the availability of substrates in animals. However, in a study of the effects of cold on substrate utilization factors such as growth, pregnancy and lactation can be removed or studied separately and therefore they will not be considered here. Reviews on the effects of growth and reproduction on glucose utilization can be found in the literature (Ford 1965; Leng 1970; Lindsay 1970; and Lindsay 1971). However, the effects of nutritional status can not be separated from the effects of cold on substrate utilization and hence a brief consideration will be given to this topic as it relates to the theme of the thesis.

Nutritional status and substrate availability

Substrate availability and blood levels are determined by the nutritional status of an animal. In a recent review, Bowden (1971) related the nutritional status of ruminants to the concentrations of free fatty acids (FFA) and ketones in the blood. The concentration of plasma FFA

in sheep increases before feeding, decreases to a minimum 2 to 4 h after eating (Annison 1960) and then gradually increases until the next feeding (Russel et al 1967). Variations in plasma FFA levels in cows related to feeding times have been observed by Hartmann and Lascelles (1965) and Kronfeld (1965). Furthermore with prolonged starvation the concentration of FFA and ketones continues to increase whereas glucose concentration decreases (Patterson et al 1964). The greater FFA utilization associated with fasting results in higher ketone production (Leng and West 1969; Bergman et al 1970; and Palmquist 1972). In the fed animal 78-94% of the ketones are derived from butyrate, however, the source of ketone production is shifted towards FFA with fasting (Leng and West 1969). Ketones appear to enhance FFA oxidation (Wakil and Bressler 1962) and/or depress FFA mobilization (Menahan et al 1966).

Bergman and coworkers (1968) calculated that the glycerol released with the mobilization of FFA provides about 25% of the estimated glucose requirement of fasted sheep. The remainder of the glucose needs during fasting are thought to be supplied largely from amino acids (Bergman 1973).

The fed ruminant derives the majority of its daily energy requirements from absorbed volatile fatty acids (Bergman et al 1965). During times of undernutrition or fasting FFA mobilized from body stores largely replace

the role of the volatile fatty acids. The supply of glucose precursors to meet glucose requirements is shifted from propionate and absorbed amino acids to glycerol and amino acids mobilized from body stores (Bergman 1973). Likewise the amino acid supply is shifted from absorption from the gut to mobilization from body protein stores.

c. Substrate Utilization for Cold Induced Heat Production

Graham et al (1959) and Blaxter and Wainman (1961) estimated the quantity of fat, protein and carbohydrate dissimilated completely in ruminants exposed to different temperatures from nitrogen metabolism, methane production, oxygen consumption and non-protein respiratory quotients. They concluded that the source of additional heat in sheep (Graham et al 1959) and cattle (Blaxter and Wainman 1961) exposed to temperatures below their critical temperature was entirely increased fat catabolism. Similar estimates based on respiratory quotients led Kayser (1937) and Pagé and Chénier (1953) to conclude that fat was used exclusively for the increased heat production of rats exposed to acute or chronic cold.

The exclusive use of fat for cold induced heat production in the rat was widely accepted for a time but was questioned by Masoro in 1966. Liver glycogen reserves in the rat are rapidly depleted on initial exposure to cold (Masoro et al 1954). Depocas and Masironi (1960)

using U-^{14} C-glucose found that a similar proportion of the CO_2 (approximately 20-22%) was derived from glucose in fed rats whether they were exposed to warm, or acute or chronic cold. This reveals that the increase in glucose metabolism in rats exposed to cold is proportional to the increase in total catabolism and hence probably heat production. Although a smaller proportion of the CO_2 is derived from glucose in the fasted rat a similar response to that found in fed rats was observed with exposure to cold (Depocas 1962).

The participation of glucose in cold induced heat production in the rat is further supported by the enzyme changes observed during cold adaptation of rats by Nakagawa and Nagai (1971). Within twelve hours of cold exposure they observed in rat liver an increased activity of phosphoenolpyruvate carboxykinase and serine dehydratase, two key gluconeogenic enzymes. In contrast key glycolytic and lipogenic enzymes in the liver were found to decrease in activity within 72 h. The glycolytic enzymes in muscle increased. These enzyme changes suggest that increased gluconeogenesis in response to cold supplies glucose as a source of energy for shivering muscle (Nakagawa and Nagai 1971).

Protein catabolism also increases in rats upon cold exposure which is evidenced by the increased urinary

nitrogen excretion with initial (You et al 1950) and prolonged cold (Young and Cook 1955). On the basis of increased urea concentrations in the liver and blood plasma of cold exposed rats, Nakagawa and Nagai (1971) suggested that proteins or amino acids are utilized as substrates for cold induced gluconeogenesis even in animals on a carbohydrate rich diet.

After reviewing the literature on rats, Masoro (1966) came to the conclusion that fat is not the exclusive fuel, nor is it preferentially utilized for the increased heat production associated with cold exposure, but rather that the utilization of all available substrates is increased.

The question arises as to why the RQ value may be a poor indicator of substrate utilization in vivo. In calculating the contribution of substrates to heat production from nitrogen metabolism and non-protein respiratory quotients (RQ's) it is assumed that all the substrates are completely oxidized which may not be the case. For example, via the Cori cycle glucose released from the liver is catabolized to lactate by muscle with the release of energy. The lactate is then returned to the liver where it is resynthesized to glucose. The increased glucose utilization with activation of the Cori cycle is not reflected in RQ's since it is an anaerobic process with no loss of carbon dioxide. Glucose could also be converted to pyruvate by anaerobic

glycolysis and hence to OAA which requires the uptake of CO_2 and therefore lowers RQ. If OAA levels are increased in the body, which they most likely are in times of increased metabolism, then RQ would be decreased as a result. However, this decrease would be interpreted as increased fat metabolism with no recognition being given the increased glucose utilization. As well, interpretation of RQ's do not account for the interconversion of substrates. For example, if glucose is converted to fat this would increase RQ whereas amino acids contributing to gluconeogenesis would depress uncorrected RQ's. If all the nitrogen from the amino acids contributing to gluconeogenesis are excreted in the urine then the interconversion can be accounted for by the use of non-protein RQ's. However there may be a substantial amount of nitrogen recycling in animals, especially in ruminants. Thus although RQ's may be good indicators of gross changes within the animal, e.g. depletion of fat stores, they have limited use as indicators of substrate utilization in vivo.

As previously mentioned, it was concluded on the basis of RQ's that the additional heat of sheep (Graham et al 1959) and cattle (Blaxter and Wainman 1961) exposed to cold is entirely fat catabolism. The use of RQ's for sheep and cattle as the sole basis for this conclusion, as for the rat may be invalid. Patterson et al (1964), Bost and Dorleac (1965) and Halliday et al (1969) have observed higher plasma FFA concentrations in sheep exposed to cold. Calvert et al (1972) found that hypothalamic cooling in an ox increased plasma

FFA concentration. These results support the conclusion that fat is an important substrate for cold thermogenesis. However, Halliday et al (1969) also found higher levels of blood glucose in sheep held at 8 than at 30°C. Acute exposure of these sheep caused an initial sharp rise in glucose concentration subsequently followed by a rapid decline. The deline may have been due to the depletion of the limited glycogen stores. Karihaloo et al (1970) found that the glucose concentration increased as temperature decreased in overwintered ewes. Thompson et al (1972) suggested that FFA in cattle may not be the only substrate oxidized in the cold.

Although the information on substrate utilization for cold induced thermogenesis in ruminants is limited, available results suggest that substrates other than fat may be used. Since ruminants depend on endogenous synthesis of glucose to meet their glucose requirements, they may be limited in the amount they can supply to an increased demand imposed by cold or they may produce glucose at the expense of other body tissues. Bergman (1973) suggested that the supply of glucose precursors and the organs that synthesize glucose could be limiting factors for the animal's overall productivity and even for its survival. A more direct method than the use of RQ, such as radioisotope tracer studies, should be considered for the assessment of the contribution of various substrates to cold induced heat production in ruminants.

MATERIALS AND METHODS

In order to determine the relative participations of glucose and FFA in cold induced thermogenesis in ruminants, continuous infusion trials using isotopically labelled glucose and palmitate were run on fed and fasted sheep exposed to acute and chronic warm and cold temperatures. Trials were run on fasted sheep to accentuate the role of body stores and/or endogenous synthesis of energy substrates. The study was done at the Environmental Laboratory at the University of Alberta.

A. Experimental Animals

Six mature Lincoln ewes were selected from the University flock on the basis of their similarity and willingness to accept experimental conditions imposed upon them. Throughout the study the fleece length of the sheep was maintained at less than 5 mm by close clipping every ten days. In addition each sheep was closely clipped 3 to 4 days prior to its use in a trial. The sheep were kept in metabolism crates under continuous light. Three of the sheep were "warm conditioned" in a chamber (5 X 9m) maintained at 18 $\pm 2^{o}C$ while the other three were "cold conditioned" in a chamber (3 X 4m) maintained at $-2 \pm 2^{o}C$. The sheep were kept in these chambers a minimum of 4 weeks prior to the initiation of the trials. However during this preliminary period the sheep were exposed and trained to the experimental conditions.

The warm conditioned sheep received 1000 - 1500 g and the cold conditioned sheep 1500 - 2000 g of alfalfa pellets daily. The rations were adjusted within these ranges according to the loss or gain of weight of the animals. The chemical analysis of the feed is given in Appendix A. Both groups of sheep were fed once daily at 1700 h.

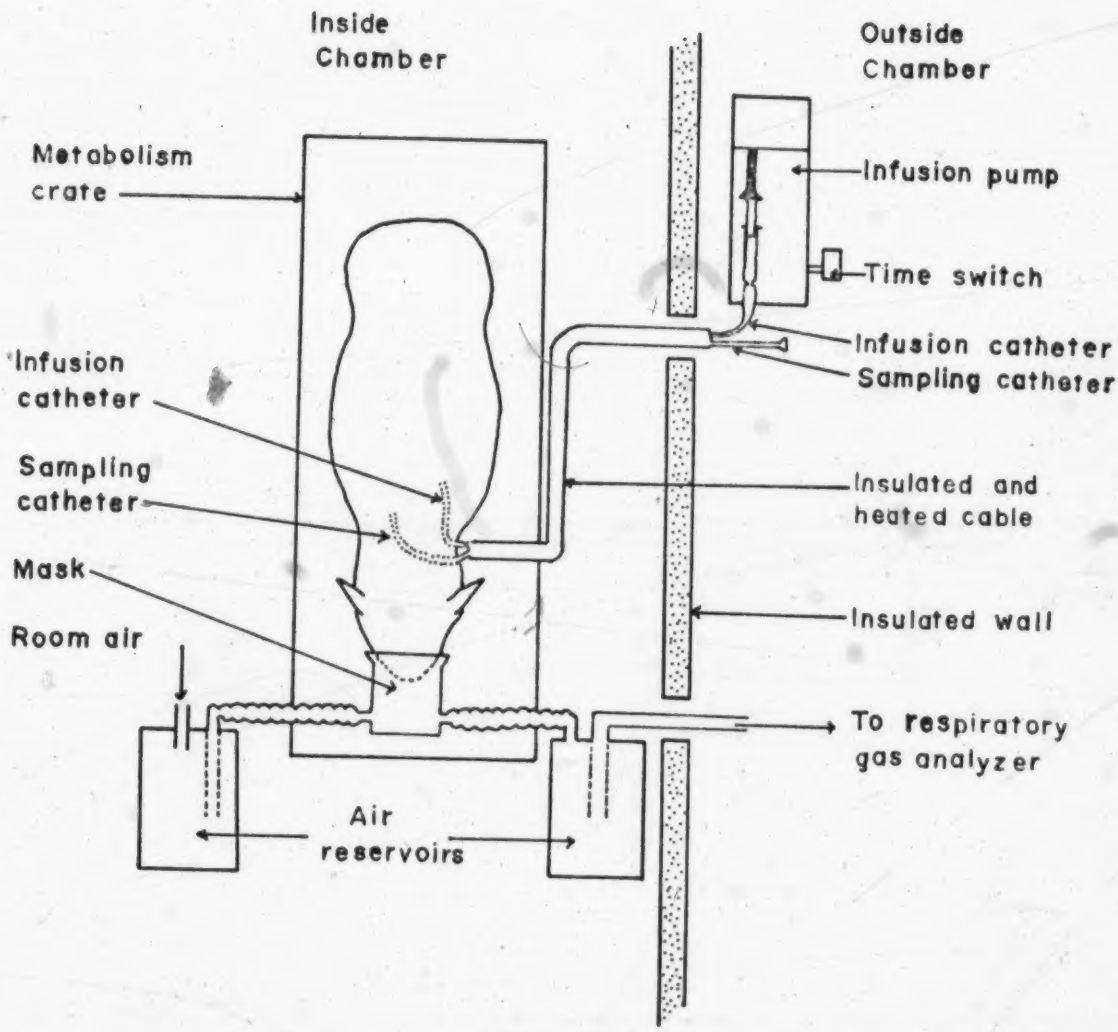
In the present study "fed" sheep refers to sheep which were fed the day preceding a trial and therefore had eaten 11-12 hours prior to the trial. "Fasted" sheep refers to sheep which were not fed the day preceding a trial and therefore were without food 35-36 hours prior to the trial.

B. Experimental Procedures

Approximately 14 hours preceding a trial, a polyethylene infusion catheter (PE-190 Intramedic, Clay Adams) was inserted 35 cm into the left jugular vein and a similar sampling catheter 15 cm into the right jugular vein. The sheep was then placed in a metabolism crate within the experimental chamber (3 X 4 m) at a temperature of 18 $\pm$ 2°C.

Figure 1 illustrates the arrangement of the sheep and associated apparatus during a trial. The infusion and sampling catheters which extended to the outside of the chamber were wrapped with heating tape (Wrap-on Co.

Figure 1. Arrangement of Sheep and Apparatus During a Trial

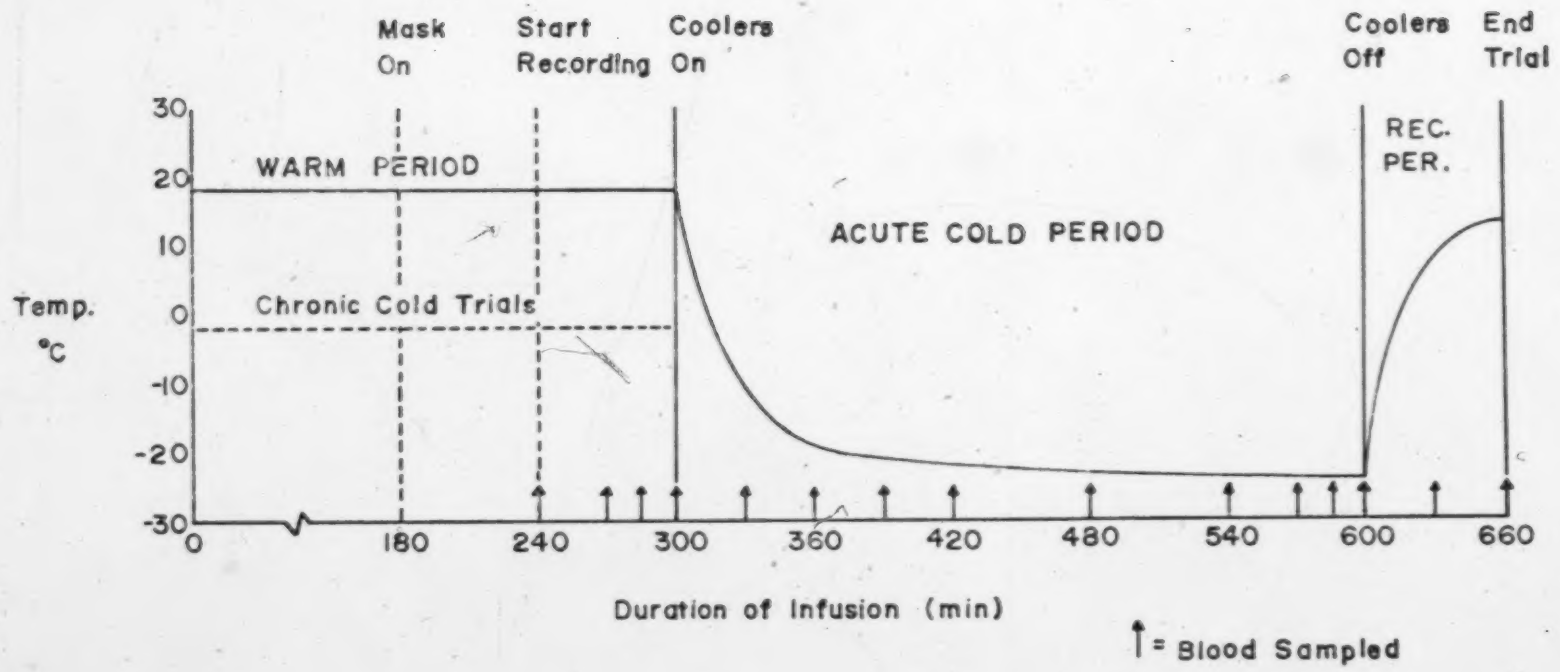


Manuf., Chicago, U.S.A.) and fiberglass insulation. Continuous infusions were given via the catheter inserted in the left jugular with a Harvard Infusion Pump (15703F Harvard Apparatus Co. Inc.) which was connected to an automatic electric timer set to start the pump at 0500 h the morning of a trial. Between 0800 h and 0830 h a respiration mask was placed on the sheep and connected via an insulated and heated rubber hose to a respiratory gas analyser. Blood samples were taken via the catheter inserted in the right jugular.

The sequence of events for the trials is illustrated in Figure 2. The experimental chamber was maintained at 18 $\pm 2^{o}$C for an additional five hours after the start of an infusion. This five hour warm period was followed by a five hour acute cold period during which the coolers were turned on and the room was cooled as quickly as possible to -24 $\pm 2^{o}$C. After the five hour acute cold period the coolers were turned off and the chamber doors opened to allow the warmer external air to exchange with the cold air in the chamber. This one hour period is referred to as the recovery period. Respiratory gases were monitored from the fourth hour of the warm period to the end of the recovery period. Blood samples were also taken during this time as indicated on Figure 2.

Additional trials of short duration were run on

Figure 2. Sequence of Events in Trials



the cold conditioned sheep. These trials were similar to those described above except that the temperature of the experimental chamber was maintained at -2 ±2°C rather than 18 ±2°C during the initial period and these trials were terminated after 5 hours of infusion.

C. Experimental Design

The experimental design is outlined in Table 2. There were a total of six sheep; three warm conditioned and three cold conditioned. These sheep were either in a fed or fasted state for a trial. Trials in which continuous infusions of glucose or palmitate were used are referred to as glucose and palmitate trials respectively. Glucose and palmitate trials of long duration, that is where the chamber temperature was changed from 18 to -24°C after the fifth hour, were made on all sheep in both fed and fasted states. The exposures to 18 and -24°C in each of the trials of long duration are shown separately in Table 2 and in the following tables of similar format. Glucose and palmitate trials in which the chamber temperature was -2°C, that is the trials of short duration, were made only on the cold conditioned sheep.

In this study an acute exposure refers to the exposure of an animal for 5 to 24 hours to a temperature other than the one to which it was conditioned. A chronic exposure refers to an animal in the same temperature it

Table 2. Experimental Design (The format used in this table has also been used to present results of this study.)

Sheep			Glucose trials			Palmitate trials			[a]Combined		
			[b]18	-2	-24	18	-2	-24	18	-2	-24°C
Fed	Warm conditioned	[c](18°C)	[d]3		3	3		3	6		6
	Cold conditioned	(-2°C)	3	3	3	3	3	3	6	6	6
Fasted	Warm conditioned	(18°C)	3		3	3		3	6		6
	Cold conditioned	(-2°C)	3	3	3	3	3	3	6	6	6

[a] Combined glucose and palmitate trials, in this case simply the total number of glucose and palmitate trials. In subsequent tables means of values for both types of trials are included in these columns.

[b] Temperature at which measurements were made.

[c] Temperature to which sheep were conditioned for a minimum of 4 wk prior to a trial.

[d] Number of exposures, one exposure per sheep.

was kept for a minimum of 4 weeks. A minimum of 3 weeks was allowed between acute exposure of sheep to minimize the effects of previous acute exposures on subsequent trials.

D. Infusates

U-^{14}C-glucose (CFB-2 Amersham/Searle Corp.) was dissolved in 0.9% sterile saline containing 0.01% glucose carrier to give a specific activity of approximately 2uc/ml. This solution was infused at a rate of 0.075 ml/min which resulted in an infusion rate of radioactivity of approximately 0.15 uc/min.

1-^{14}C-palmitate (NEC-075 New England Nuclear) was dissolved in ethanol (250 uc/ml). Approximately 0.5 ml of this solution was added very slowly, while stirring, to 10 ml of plasma which had been obtained from the sheep when the jugular catheters were inserted (approximately 12 h before the infusate was used). This solution was then diluted slowly with 0.9% sterile saline to give a final concentration of approximately 1.8 uc/ml. The solution was infused into the sheep at a rate of 0.075 ml/min. The rate of isotope infusion was therefore approximately 0.12 uc/min.

E. Blood Sampling

The arrows in Figure 2 indicate the times at which 10 ml blood samples were taken. The sampling catheter was flushed with heparinized saline immediately before and

after taking each sample. Samples were taken with heparinized disposable syringes and transferred to centrifuge tubes. Two small aliquots were taken for micro-hematocrit determination and the remaining sample was immediately centriguted at 2000 X G for 10 minutes. The plasma was removed with Pasteur pipettes and immediately frozen at -25°C for subsequent analysis.

F. Analytical Procedures

a) Respiratory gas analysis

Oxygen, carbon dioxide and isotopically labelled carbon in the expired gas of the experimental sheep were continuously monitored during the trials using instrumentation for animal calorimetry similar to that described by Young (1974). The mean respiratory gas values measured 7.5 minutes before to 7.5 minutes after blood sampling were used in this thesis.

b) Glucose concentration

The plasma glucose concentration in the glucose trials was determined enzymatically by the Glucostat Method (Worthington Biochemical Corporation, Freehold, New Jersey).

c) Specific activity of glucose

Glucose was isolated from the plasma as the glucose pentaacetate derivative for the determination of

specific activity by liquid scintillation spectrometry as described by Jones (1965) with some modifications. See Appendix B1 for the modified procedure.

d) Separation of free fatty acids (FFA)

The FFA were separated from the plasma obtained in palmitate trials by a modified Dole and Meinertz (1960) procedure (see Appendix B2). Individual FFA were separated as methyl esters (see Appendix B3 for preparation of methyl esters) by gas chromatography at 220°C with a 12 foot glass column packed with acid washed Chromosorb W (01-0136 Supelco, Inc.) coated with 10% Silar-5CP (08270 Applied Sciences Laboratories, Inc.). The concentration of individual FFA was determined by flame ionization in a Bendix Gas Chromatograph 2500 (2532-3 Canadian Dynamics Ltd.) and results were recorded on a Photovolt Recorder (Microcord 44, Photovolt Corp.) and by a Digital Integrator (Vidar 6300-02 Canadian Dynamics Ltd.).

e) Specific activity of palmitate

Palmitate was collected after it passed through the gas chromatograph by placing cigarette filters (V-Master Ltd.) over one channel of the gas chromatograph column splitter at the appropriate time. The filters were previously soaked in 5% silicone oil (710 Fluid, Dow Corning Silicones Inter-America Ltd.) pentane mixture and then dried. The radioactivity in the filter was

determined by liquid scintillation (Hammarstrand et al 1969).

G. Calculations

a) Respiratory quotient (RQ)

The respiratory quotients of the sheep were calculated by dividing the mean carbon dioxide production over a fifteen minute interval by the mean oxygen consumption over the same interval.

$$RQ = \frac{CO_2 \text{ production (ml/min)}}{O_2 \text{ consumption (ml/min)}}$$

b) Heat production (Hp)

Approximately 4.892 calories are used for each ml of oxygen consumed (McLean 1970). Therefore the following equation was used to calculate the heat production of the sheep from the mean oxygen consumption over each 15 minute interval.

$$\underset{(cal/min/kg)}{Hp} = \frac{O_2 \text{ consumption (ml/min)} \times 4.892 \text{ (cal/ml)}}{\text{Body wt (kg)}}$$

c) Specific activity (SA)

"The specific activity of a radioisotope sample is the amount of radioactivity per unit amount of material." (Wang and Willis 1965). The following formula was used in this study.

$$\underset{(uc/mg\ C)}{SA} = \frac{\text{uc of radioactivity}}{\text{mg of carbon}}$$

RESULTS

For ease of reading and interpretation the results have been presented in the following order:

1) General observations.

2) Graphic presentation of a typical trial.

3) Each parameter measured has been summarized and presented in a table (Tables 3-8) with a standardized format. Statistical summaries are also given in these tables.

4) Details of each trial and all measurements taken during the trial are presented in the Appendices (C1 - C33).

5) A number of parameters were calculated from the results. The calculations used to derive these parameters are therefore presented.

6) Each derived parameter has been summarized and presented in table form (Tables 9 - 13).

7) An overall summary of the results and derived parameters is presented.

A. General Observations

At the commencement of the study the body weights of the sheep ranged from 65 to 85 kg. The weights of the warm conditioned sheep were quite variable ($\pm$4 kg) however the average net change for the experimental period (Jan. 5 -

June 5, 1972) was less than 2 kg. Despite a higher level of feeding the cold conditioned sheep lost an average of 10 kg body weight over the 5 month period. The sheep lost as much as 3-4 kg of body weight when fasted. The cold conditioned sheep were slow to recover this weight and often did not return to their pre-trial weight. Despite an increased offering of ration after sheep were fasted, the cold conditioned animals often left feed and body weight tended to be maintained at a new lower level. One of the cold conditioned sheep (#1330) refused to eat any food after a trial in which she was fasted. She was removed from the cold conditioning chamber however she died approximately one week later. Because of the loss of this animal 1 glucose and 3 palmitate trials could not be completed.

Generally cold conditioned sheep were more irritable, which was shown by gross body movement and tossing of the head, than were the warm conditioned animals. This difference was particularly noticeable towards the end of trials where the exposure temperatures were 18 and -2°C. Similarly the animals when fasted tended to be more irritable than when in a fed state. The animals usually changed their behavioural activity when the coolers were turned on for the acute cold exposure (-24°C). For example animals which were restless towards the end of the initial period at 18°C ceased their head tossing activity once the

coolers were turned on and remained fairly quiet except for the occassional scraping of the floor of the metabolism crate with their fore foot.

Figure 3 and 4 illustrate the results of a typical trial. The results shown are for a warm conditioned sheep (#1090) in a fed state. The measurements were made during an initial period in which the chamber was at 18°C and a subsequent acute cold period in which the chamber was rapidly cooled to -24°C. Measurements were also taken in the recovery period during which the chamber temperature increased to 14°C.

Oxygen consumption, carbon dioxide production, hematocrit, plasma glucose concentration, *specific activity of CO_2 from glucose, plasma palmitate concentration and *specific activity of CO_2 from palmitate all increased whereas the specific activity of plasma glucose decreased when the temperature of the chamber was lowered. In most cases the measurements obtained during the recovery period tended to return towards those obtained in the pre-acute cold period.

Only those measurements obtained between 240 and 300 minutes and between 570 and 600 minutes, illustrated by A and B in Figures 3 and 4, have been used in the tables of results (Tables 3-8). The means and standard errors of the means presented in Tables 3-8 are based on the measurements from three trials made on different animals except where

* Specific activity of CO_2 measured during the infusion of U-^{14}C-glucose or 1-^{14}C-palmitate.

Figure 3. Results of a Typical Trial in which a fed warm conditioned sheep (#1090) was exposed to different environmental temperatures. All parameters were taken from Appendix C2. Measurements taken during time periods A and B are used in the summary tables (Tables 3-8).

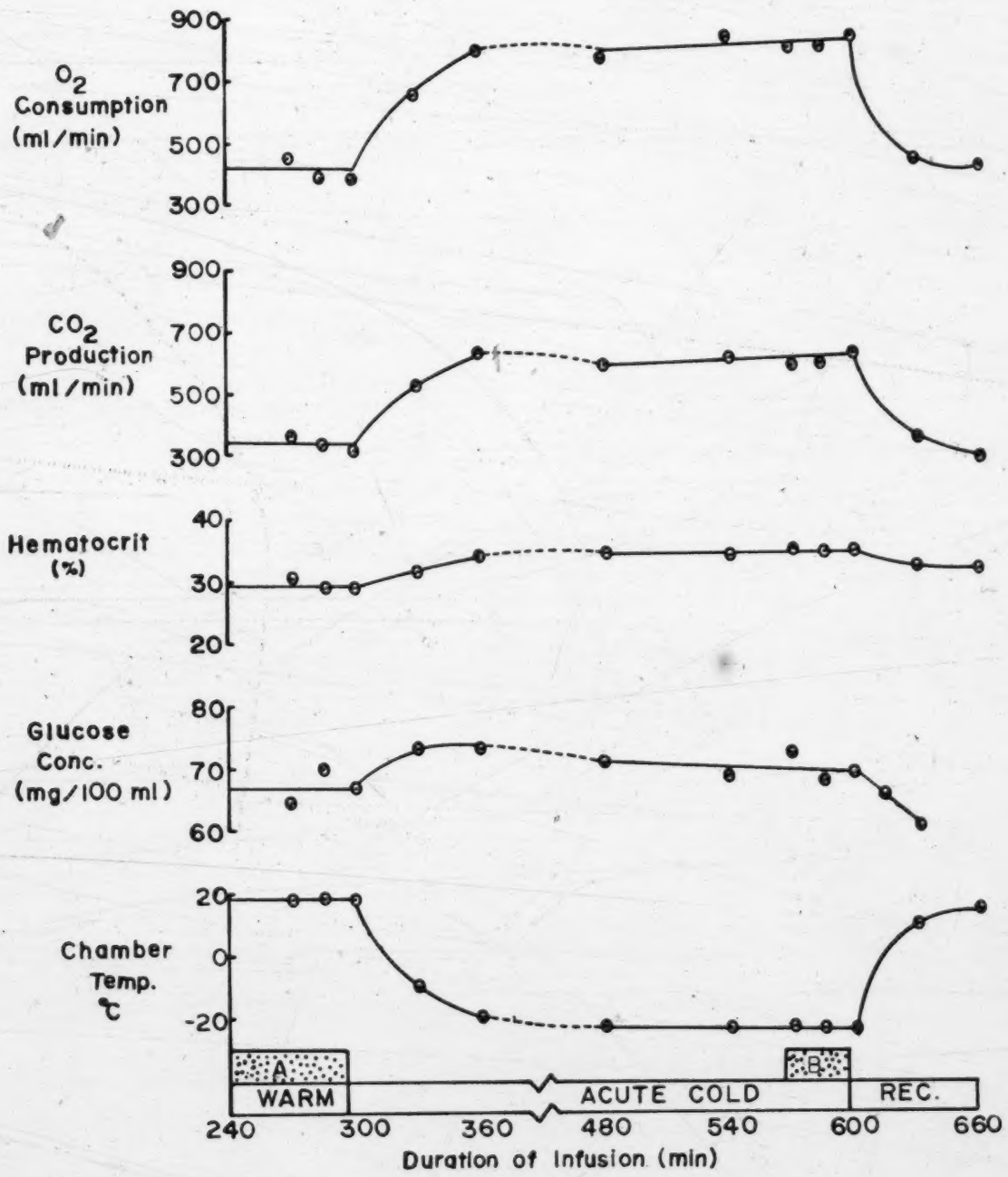
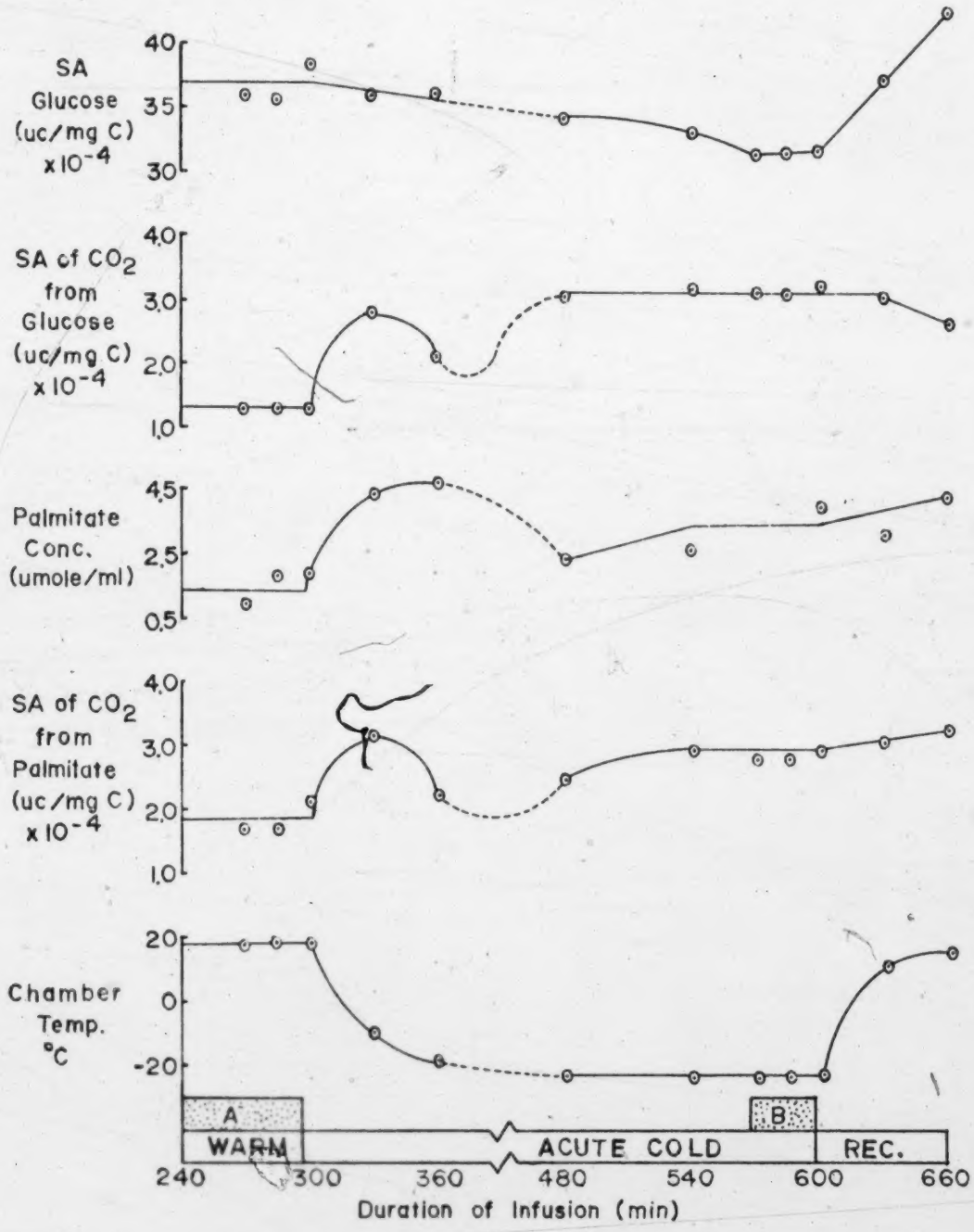


Figure 4. Results of a Typical Trial (cont'd) in which a fed warm conditioned sheep (#1090) was exposed to different environmental temperatures. All parameters were taken from Appendix C2 except for palmitate parameters which were taken from the corresponding palmitate trial, Appendix C20.



only two animals were available and hence only two trials were completed. The results from the recovery period (Figure 1) are not summarized (Tables 3-8). The measurements taken during the recovery period are in Appendices C1-C33. The results from the recovery period are drawn upon for the discussion where they assist in interpretation.

A standard format has been used to present the results. For example, Table 3 p 42 is a summary of the hematocrits obtained from the glucose and palmitate trials. The hematocrits have been grouped in columns according to the exposure temperatures at which measurements were taken. The table is divided into rows according to whether the sheep were fed or fasted and further subdivided according to whether they were warm or cold conditioned. For example, from Table 3 the mean hematocrit for fasted warm conditioned sheep chronically exposed to 18°C was 32.9%. Where possible the results from glucose and palmitate trials have been combined in the last three columns of the tables to give an overall mean. Statistical comparisons of the means from palmitate and glucose trials were made with the Student's t test (Steel and Torrie 1960). A "c" beside the overall mean in the combined columns signifies a statistically significant difference ($P<0.05$) was found to exist between means from the glucose and palmitate trials.

Five statistical comparisons were made for the results summarized in Tables 3-8. These comparisons were

made for the sheep in both fed and fasted states.

Comparisons I were between the warm and cold conditioned sheep at their conditioning temperature (18 and -2°C respectively) which is a comparison of chronic warm exposure and chronic cold exposure.

Comparisons II were between warm and cold conditioned sheep when exposed to 18°C. As 18°C was the conditioning temperature for the warm conditioned animal this was a chronic exposure situation whereas to the sheep previously conditioned to -2°C it was an acute exposure.

Comparisons III involves only warm conditioned sheep exposed to 18 and -24°C. The measurements obtained at the two exposure temperatures were compared.

Comparisons IV involves only cold conditioned sheep exposed to 18 and -24°C. The measurements obtained at these two exposure temperatures were compared.

Comparisons V were between warm and cold conditioned sheep acutely exposed to severe cold (-24°C).

Comparisons I, II and V were tested for statistical significance by the Student's t test for comparisons of unpaired means whereas comparisons III and IV were tested for statistical significance by Student's t test for paired means (Steel and Torrie 1960).

B. Hematocrits

Hematocrits obtained during the glucose and palmitate trials are summarized in Table 3. The hematocrits measured during corresponding glucose and palmitate trials should be similar and therefore the combined results were considered.

The hematocrits of fed cold conditioned sheep chronically exposed to -2°C were significantly greater ($P < 0.05$) than those of the sheep chronically exposed to 18°C. Fasting caused the hematocrits of the warm conditioned sheep to increase slightly and hematocrits of the cold conditioned sheep to decrease such that the differences between hematocrits of the fasted sheep were not significant.

The effect on hematocrits of exposing cold conditioned sheep to warm temperatures can be seen by comparing the hematocrits of cold conditioned sheep acutely exposed to 18°C with those of warm conditioned sheep chronically exposed to 18°C. In the fed animals, the hematocrits are significantly ($P < 0.05$) higher in the cold conditioned than in the warm conditioned animals. These differences tend to disappear with fasting.

The hematocrits increased in all cases when the sheep were acutely exposed to -24°C. The differences between warm and cold conditioned sheep exposed to -24°C were insignificant in both fed and fasted states.

Table 3. Hematocrits (%) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep			Glucose trials			Palmitate trials			[b]Combined results		
			18	-2	-24	18	-2	-24	18	-2	-24°C
Fed	Warm conditioned	(18°C)	[a]30.1 ±0.7		35.3 ±0.4	29.8 ±1.0		33.2 ±1.8	30.0 ±0.6		34.2 ±0.9
	Cold conditioned	(-2°C)	34.0 ±0.8	34.8 ±1.5	37.2 ±1.4	31.1 ±0.5	33.4 ±1.4	34.8 ±1.3	[c]32.5 ±0.8	34.1 ±0.8	36.0 ±1.0
Fasted	Warm conditioned	(18°C)	32.9 ±1.1		37.3 ±1.1	31.2 ±1.2		32.7 ±0.8	32.0 ±0.8		[c]35.0 ±1.2
	Cold conditioned	(-2°C)	32.6 ±1.4	32.0 ±0.4	36.2 ±1.9	40.7 ±0.1	29.6 ±2.0	43.3 ±0.1	36.7 ±1.9	30.8 ±0.8	39.8 ±2.2

Comparisons		Glucose trials	Palmitate trials	Combined results
I Warm vs. cold conditioned at conditioning temp.	Fed	*	-	*
	Fasted	-	-	-
II Warm vs. cold conditioned at 18°C	Fed	*	-	*
	Fasted	-	*	-
III Warm conditioned at 18 vs. -24°C	Fed	*	-	*
	Fasted	*	-	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*	*
	Fasted	-	*	*
V Warm vs. cold conditioned at -24°C	Fed	-	-	-
	Fasted	-	*	-

[a] Mean ± standard error of the mean

[b] Glucose & palmitate trials

[c] Sign. diff. ($P < 0.05$) between glucose & palmitate trials

* Sign. diff. ($P < 0.05$)

Some statistically significant differences existed between hematocrits from glucose and palmitate trials. The reason for these differences is not readily apparent.

In brief. Both acute and chronic cold increased the hematocrits of fed sheep but had little effect on fasted sheep. Fasting generally increased the hematocrits of warm conditioned sheep and decreased the hematocrits of cold conditioned sheep. The cold conditioned sheep had higher hematocrits when acutely exposed to 18°C than the warm conditioned sheep chronically exposed to 18°C. Differences between cold and warm conditioned animals became smaller when animals were exposed to acute cold or when the animals were fasted.

C. Respiratory Quotients

Table 4 summarizes the respiratory quotients (RQ) obtained in the glucose and palmitate trials. The RQ values have not been corrected for protein catabolism.

The RQ's of fasted sheep were lower than those of fed sheep in all situations.

The chronic exposure of cold conditioned sheep gave lower RQ's than the chronic exposure of warm conditioned sheep, however the differences were statistically significant only when the sheep were fasted.

Table 4. Respiratory Quotients of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose trials			Palmitate trials			[b]Combined results		
		18	-2	-24	18	-2	-24	18	-2	-24°C
Fed	Warm conditioned (18°C)	[a]0.82 ±0.02		0.76 ±0.02	0.77 ±0.06		0.76 ±0.04	0.79 ±0.03		0.76 ±0.02
	Cold conditioned (-2°C)	0.79 ±0.06	0.76 ±0.02	0.75 ±0.02	0.76 ±0.02	0.73 ±0.00	0.70 ±0.02	0.77 ±0.03	0.74 ±0.01	0.73 ±0.02
Fasted	Warm conditioned (18°C)	0.71 ±0.02		0.72 ±0.00	0.72 ±0.02		0.67 ±0.01	0.72 ±0.01		0.69 ±0.01
	Cold conditioned (-2°C)	0.74 ±0.02	0.63 ±0.01	0.69 ±0.03	0.73 ±0.03	0.67 ±0.00	0.69 ±0.01	0.74 ±0.01	0.65 ±0.01	0.69 ±0.01

Comparisons		Glucose trials	Palmitate trials	Combined results
I Warm vs. cold conditioned at conditioning temp.	Fed	-	-	-
	Fasted	*	-	*
II Warm vs. cold conditioned at 18°C	Fed	-	-	-
	Fasted	-	-	-
III Warm conditioned at 18 vs. -24°C	Fed	-	-	-
	Fasted	-	-	-
IV Cold conditioned at 18 vs. -24°C	Fed	-	-	-
	Fasted	-	-	*
V Warm vs. cold conditioned at -24°C	Fed	-	-	-
	Fasted	-	-	-

[a] Mean ± standard error of the mean

[b] Glucose and palmitate trials

* Sign. diff. ($P < 0.05$)

The RQ's of cold conditioned sheep exposed to 18°C were not significantly different from those of the warm conditioned sheep exposed to 18°C.

The RQ's were generally lower in sheep acutely exposed to -24 than at 18°C. The warm conditioned sheep had slightly higher RQ's than the cold conditioned sheep during acute cold exposures (-24°C) when fed however, the difference was negligible when fasted.

In brief. The chronic cold exposure of sheep resulted in lower RQ's, especially in fasted animals, than warm exposure of sheep. Cold conditioning had little effect on RQ's measured at 18°C. Acute exposure to -24°C and/or fasting resulted in lower RQ's than observed at 18°C or in fed animals respectively.

D. Heat Productions

Heat productions measured during the glucose and palmitate trials are summarized in Table 5. Similar results were obtained in most cases for both the palmitate and glucose trials and therefore the combined results were considered.

The cold conditioned sheep at -2°C had significantly higher ($P < 0.05$) heat productions than the warm conditioned sheep at 18°C in both fed and fasted states.

Table 5. Heat Productions (cal/min/kg) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2, and -24°C

Sheep			Glucose trials 18	Glucose trials -2	Glucose trials -24	Palmitate trials 18	Palmitate trials -2	Palmitate trials -24	[b]Combined results 18	Combined results -2	Combined results -24°C
Fed	Warm conditioned	(18°C)	[a]25.5 ±3.1		54.2 ±2.4	23.7 ±0.2		45.5 ±6.5	24.6 ±1.4		49.8 ±3.6
	Cold conditioned	(-2°C)	33.2 ±2.7	34.4 ±1.3	55.6 ±4.0	28.2 ±1.7	35.9 ±4.8	47.7 ±1.9	30.7 ±1.8	35.2 ±1.4	51.7 ±2.7
Fasted	Warm conditioned	(18°C)	22.9 ±3.1		49.1 ±4.0	20.0 ±2.3		52.5 ±0.8	21.5 ±1.8		50.8 ±2.0
	Cold conditioned	(-2°C)	25.3 ±0.5	42.5 ±3.2	49.1 ±3.7	30.6 ±0.9	35.0 ±3.8	53.9 ±2.9	[c]27.9 ±1.2	38.8 ±2.4	51.5 ±2.4

Comparisons		Glucose trials	Palmitate trials	Combined results
I Warm vs. cold conditioned at conditioning temp.	Fed	-	-	*
	Fasted	*	*	*
II Warm vs. cold conditioned at 18°C	Fed	-	-	*
	Fasted	-	*	*
III Warm conditioned at 18 vs. -24°C	Fed	*	*	*
	Fasted	*	*	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*	*
	Fasted	*	*	*
V Warm vs. cold conditioned at -24°C	Fed	-	-	-
	Fasted	-	-	-

[a] Mean ± standard error of the mean
[b] Glucose & palmitate trials
[c] Sign. diff. ($P < 0.05$) between glucose & palmitate trials
* Sign. diff. ($P < 0.05$)

Both fed and fasted cold conditioned sheep acutely exposed to 18°C had significantly higher ($P < 0.05$) heat productions than the respective warm conditioned sheep at 18°C.

The heat productions of sheep exposed to acute cold (-24°C) were roughly double those observed at 18°C. There were no statistically significant differences observed in heat productions at -24°C which were due to conditioning.

In brief. Both acute and chronic exposure of sheep to cold increased the heat productions markedly. Cold conditioning of sheep increased the heat productions observed in the warm (18°C).

E. Concentrations of Plasma Glucose and Palmitate

The plasma concentrations of glucose and palmitate obtained in the glucose and palmitate trials respectively are presented in Table 6. The units used in Table 6 for expressing glucose and palmitate concentrations are those traditionally used. The palmitate concentration may be converted to mg/100 ml by multipling the umoles/ml by 25.64.

a) Concentrations of plasma glucose

None of the comparisons made between mean glucose concentrations in Table 6 were statistically significant although some may be biologically important.

Table 6. Concentrations of Plasma Glucose (mg/100 ml) and Palmitate (umole/ml) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose conc.			Palmitate conc.		
		18	-2	-24	18	-2	-24
Fed	Warm conditioned (18°C)	[a] 59.1 ±7.4		73.6 ±3.0	0.109 ±0.014		0.519 ±0.116
	Cold conditioned (-2°C)	69.7 ±10.0	74.7 ±10.9	69.9 ±5.6	0.128 ±0.009	0.268 ±0.003	0.387 ±0.083
Fasted	Warm conditioned (18°C)	68.8 ±5.9		59.9 ±6.1	0.285 ±0.018		0.870 ±0.142
	Cold conditioned (-2°C)	79.3 ±5.0	82.1 ±1.9	77.9 ±5.4	0.504 ±0.017	0.388 ±0.118	0.465 ±0.023

Comparisons		Glucose conc.	Palmitate conc.
I Warm vs. cold conditioned at conditioning temp.	Fed	-	*
	Fasted	-	-
II Warm vs. cold conditioned at 18°C	Fed	-	-
	Fasted	-	*
III Warm conditioned at 18 vs. 024°C	Fed	-	*
	Fasted	-	*
IV Cold conditioned at 18 vs. -24°C	Fed	-	*
	Fasted	-	-
V Warm vs. cold conditioned at -24°C	Fed	-	-
	Fasted	-	-

[a] Mean ± standard error of the mean

* Sign. diff. ($P<0.05$)

Sheep chronically exposed to cold had higher plasma glucose concentrations than those chronically exposed to warm in both the fed and fasted states. Fasting resulted in increased glucose concentrations during chronic exposures for both warm and cold conditioned sheep.

The concentrations of glucose were generally higher in the cold conditioned animals at 18°C than in the warm conditioned animals at 18°C when either fed or fasted. The fasted animals at 18°C had higher average glucose concentrations than the fed animals.

The average glucose concentration of fed warm conditioned sheep increased from 59.1 at 18°C to 73.6 mg/100 ml at -24°C. The same animals fasted had concentrations of 68.8 mg/100 ml in the warm and these tended to decrease to about 59.9 mg/100 ml during acute cold exposure. The acute cold exposure of cold conditioned sheep appeared to have little effect on glucose concentration. The glucose concentrations were approximately 69.8 and 78.6 mg/100 ml for fed and fasted cold conditioned sheep respectively at both 18 and -24°C.

The warm conditioned sheep had higher glucose concentrations than the cold conditioned sheep when exposed to acute cold in a fed state however the order was reversed in a fasted state. The highest plasma glucose concentrations observed during acute cold were for the fasted cold conditioned

sheep.

In brief. The differences in glucose concentrations were not statistically significant although some differences may have been biologically significant. Chronic cold appeared to increase the plasma glucose concentrations. Acute cold increased the plasma glucose concentrations in warm conditioned sheep but had little effect on cold conditioned sheep. Cold conditioning increased glucose concentrations observed in the warm. In most cases, with the exception of the acute cold exposure of warm conditioned sheep, the glucose concentrations were higher in fasted than fed sheep. The highest glucose concentrations at all exposure temperatures were recorded for fasted cold conditioned sheep.

(b) Concentrations of plasma palmitate

The concentrations of plasma palmitate are summarized in Table 6. Fasting increased the plasma palmitate concentration in all cases. The fed cold conditioned sheep had significantly ($P < 0.05$) higher palmitate concentrations at $-2^{\circ}C$ than did the fed warm conditioned sheep at $18^{\circ}C$.

The plasma palmitate concentrations in fed cold conditioned sheep acutely exposed to warm ($18^{\circ}C$) were not significantly different than those observed for warm

conditioned sheep at 18°C. In the fasted animals however, the cold conditioned sheep had significantly ($P<0.05$) higher palmitate concentrations than the warm conditioned sheep (0.28 & 0.50 umoles/ml respectively).

Acute cold exposure caused the plasma palmitate concentration to increase significantly ($P<0.05$) in all sheep with the exception of the fasted cold conditioned sheep where it decreased slightly from 0.50 to 0.47 umole/ml. Warm conditioned sheep had larger plasma palmitate concentrations during acute cold exposures than did the cold conditioned sheep in both fed and fasted states.

In brief. Chronic cold, acute cold and fasting all appear to increase the plasma palmitate concentration. However acute cold did not increase the concentration in cold conditioned fasted sheep. Under similar treatment these sheep were observed to have high glucose concentrations. It should be noted that the treatments had a much greater effect on plasma palmitate concentrations than they did on plasma glucose concentrations.

F. Specific Activities of Plasma Glucose

The specific activities of plasma glucose appearing in Appendices C1 - C18 were calculated as indicated in Materials and Methods page 33. These specific activities have been standardized and summarized in Table 7 for

Table 7. Standardized[a] Specific Activities of Plasma Glucose of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose trials 18	-2	-24°C
Fed	Warm conditioned (18°C)	[b]2.07 ±0.18		1.61 ±0.05
	Cold conditioned (-2°C)	1.29 ±0.35	0.82 ±0.10	0.84 ±0.16
Fasted	Warm conditioned (18°C)	2.23 ±0.04		1.67 ±0.15
	Cold conditioned (-2°C)	1.38 ±0.11	1.38 ±0.05	1.49 ±0.09

Comparisons		Glucose trials
I Warm vs. cold conditioned at conditioning temp.	Fed	*
	Fasted	*
II Warm vs. cold conditioned at 18°C	Fed	-
	Fasted	*
III Warm conditioned at 18 vs. -24°C	Fed	*
	Fasted	-
IV Cold conditioned at 18 vs. -24°C	Fed	*
	Fasted	-
V Warm vs. cold conditioned at -24°C	Fed	*
	Fasted	-

[a] Specific activities were standardized by dividing by the infusion rate (uc/min/kg)

[b] Mean ± standard error of the mean * Sign. diff. ($P < 0.05$)

comparative purposes. The specific activities were standardized by dividing the specific activity of plasma glucose (uc/mg of glucose C) by the microcuries infused per minute per kilogram body weight (Bartley and Black 1966).

The specific activities of plasma glucose of the cold conditioned sheep at -2°C were significantly ($P < 0.05$) lower than those of the warm conditioned sheep at 18°C in both fed and fasted states. Fasting during the chronic exposures resulted in higher specific activities in both cases.

Cold conditioned sheep acutely exposed to warm (18°C) had lower specific activities of plasma glucose than observed for warm conditioned sheep exposed to 18°C. Fasting had little effect on the specific activities of the cold conditioned sheep at 18°C.

In most trials the specific activity of plasma glucose decreased with acute cold with the exception of the fasted cold conditioned sheep which showed very little difference in specific activity at any of the exposure temperatures (18, -2 or -24°C). The cold conditioned sheep had lower specific activities during the acute cold than the warm conditioned sheep in similar states.

In brief. Both chronic and acute cold resulted in lower plasma glucose specific activities than those observed in the warm at 18°C. Cold conditioned sheep had lower specific activities than warm conditioned sheep at 18°C

and -24°C. The effect of fasting was to increase the glucose specific activity.

G. Specific Activities of Plasma Palmitate

The recoveries of isotopically labelled palmitate from plasma were quite low and there was some cross contamination of label between samples. The cross contamination of label between samples most likely occurred within the Gas-Liquid Chromatography column. Therefore an acceptable direct measure of the specific activities of plasma palmitate could not be obtained. A method for estimating the specific activity of plasma palmitate from the concentration of palmitate and the rate of infusion of isotopically labelled palmitate is described in the Derived Results section, page 62. These estimates are not included in this section of the thesis because the derivations required certain assumptions which could be questioned. They are presented in Appendices C19-C33 if required.

H. *Specific Activities of CO_2 from Glucose and Palmitate

The standardized specific activities of expired CO_2 from the glucose trials are summarized in Table 8. The method of standardization was the same as that described for the specific activity of plasma glucose.

a) Specific activity of CO_2 from glucose

The specific activities of CO_2 of the fed cold

* Specific activity of CO_2 measured during the infusion of U-^{14}C-glucose or 1-^{14}C-palmitate.

Table 8. Standardized[a] Specific Activities of CO_2 Produced by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep			Glucose trials			Palmitate trials		
			18	-2	-24	18	-2	-24
Fed	Warm conditioned	(18°C)	[b]8.5 ±1.5		16.3 ±0.8	11.6 ±3.7		16.4 ±2.2
	Cold conditioned	(-2°C)	8.9 ±1.3	8.1 ±0.4	12.3 ±2.1	10.3 ±0.7	9.6 ±0.7	14.9 ±1.5
Fasted	Warm conditioned	(18°C)	11.1 ±0.8		16.6 ±0.5	21.3 ±0.9		16.8 ±1.2
	Cold conditioned	(-2°C)	7.7 ±0.8	7.4 ±0.0	9.4 ±0.1	15.2 ±0.2	15.1 ±0.5	15.4 ±0.2

Comparisons		Glucose trials	Palmitate trials
I Warm vs. cold conditioned at conditioning temp.	Fed	-	-
	Fasted	*	*
II Warm vs. cold conditioned at 18°C	Fed	-	-
	Fasted	*	*
III Warm conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	-	-
V Warm vs. cold conditioned at -24°C	Fed	-	-
	Fasted	*	-

[a] Specific activities were standardized by dividing by the infusion rate (uc/min/kg)

[b] Mean ± standard error of the mean

* Sign. diff. ($P < 0.05$)

conditioned sheep at -2°C did not differ significantly from the fed warm conditioned animals at 18°C. The cold conditioned sheep had lower and the warm conditioned sheep had higher specific activities of CO_2 when fasted than fed. The difference between warm and cold conditioned sheep when fasted during chronic exposures was significant ($P < 0.05$).

The fed cold conditioned sheep had slightly higher specific activities than the fed warm conditioned sheep at 18°C. However, fasting decreased the specific activity of CO_2 in the cold conditioned animal and increased it in the warm conditioned animal such that the warm conditioned had a significantly higher ($P < 0.05$) specific activity at 18°C in the fasted state.

The specific activities of CO_2 observed during acute cold exposure were greater than those observed at 18°C for all treatments. During acute cold exposure the specific activities of CO_2 of the warm conditioned animals were greater than those of the cold conditioned animals. This difference was increased by fasting.

In brief. Chronic cold had very little effect on the specific activity of CO_2 from glucose in fed sheep however chronic cold caused the specific activity to decrease in fasted sheep. Cold conditioning resulted in higher specific activities at 18°C than warm conditioning for fed animals but not for fasted animals. Acute cold caused the specific activities of CO_2 from glucose to increase in all cases with

levels being higher for warm than cold conditioned sheep. Fasting generally increased the specific activity of CO_2 from glucose in warm conditioned animals and decreased it in cold conditioned animals.

b) Specific activities of CO_2 from palmitate

The standardized specific activities of CO_2 derived from palmitate are summarized in the three columns on the right of Table 8. Chronic exposure to -2°C had little effect on the specific activity of CO_2 from palmitate in the fed animal. Fasting caused a greater increase in specific activity of CO_2 from palmitate of the warm conditioned animals than of the cold conditioned during chronic exposure.

Similarly the exposure of fed cold conditioned animals to 18°C had little effect on specific activities. However, when the sheep were fasted the specific activity of CO_2 from palmitate of warm conditioned sheep was significantly greater ($P < 0.05$) than that of the cold conditioned sheep at 18°C.

The acute exposure of sheep to cold resulted in higher specific activities of CO_2 from palmitate in fed animals, a decrease in fasted warm conditioned sheep and no change in the fasted cold conditioned sheep compared to 18°C exposures. The differences in specific activity

of CO_2 from palmitate between warm and cold conditioned sheep during acute cold exposure were not statistically significant.

In brief. The specific activity of CO_2 from palmitate trials was greater in warm conditioned sheep at 18°C than in cold conditioned sheep at -2°C when the sheep were fasted. The effect of acute warm exposure on cold conditioned sheep was similar to that observed during exposure to -2°C. Acute cold exposure increased all the specific activities of CO_2 from palmitate such that there were no statistically significant differences between treatments.

The specific activities of CO_2 from palmitate were greater in the fasted treatments compared to the respective fed treatments whereas the specific activities of CO_2 from glucose were greater in the fasted treatments of warm conditioned animals but less in the fasted treatments of cold conditioned sheep compared to the respective fed treatments.

DERIVED RESULTS

In order to better explain the effect of cold on glucose and palmitate metabolism a number of calculations were made based on the results presented in the previous section. The method of each calculation is described below and then the calculations are summarized in Tables 9 - 13 using a similar format to that used for the preceding results. Following this section a small summary of the results and derived results is presented.

A. Calculations of Glucose Parameters

The glucose parameters calculated include:

Glucose flux ..... (1)
% Glucose oxidized ..... (2)
% CO_2 from glucose ..... (3)
Glucose oxidation rate ..... (4)
% Heat production from glucose . (5)

Interpretation of results obtained from the continuous infusion techniques used in sheep have relied on relatively simple mathematical treatment (Leng 1970). For example, glucose flux per unit body weight was calculated using the following formula:

$$\text{Glucose Flux (mg/min/kg)} = \frac{\text{Infusion rate (uc/min)}}{\text{SA of plasma glucose (uc/mg)}} \times \frac{1}{\text{Body wt (kg)}} \quad \ldots (1)$$

where uc = microcuries

SA = specific activity

The estimate of glucose flux varies directly with the rate of infusion of the isotope and inversely with the specific activity of plasma glucose (Leng 1970). The terms flux, irreversible loss, turnover rate, transfer rate, utilization rate, entry rate, inflow-outflow rate and renewal rate, are considered to be measures of the same basic phenomenon (Leng 1970). In this dissertation the term flux has been used.

The validity of the estimates of parameters of glucose metabolism is dependent upon the assumption that all glucose entering into the system instantaneously mixes in the sample pool and also that relatively steady-state conditions are maintained throughout the measurement period (Baker et al 1959). The specific activity will "plateau" if the rate of entry of isotope into the system equals the rate of exit. It is this asymptotic value for specific activity which should be used in the calculation of glucose parameters since it indicates steady state conditions. Leng et al (1967) and White et al (1969) found that in sheep the specific radioactivity of plasma glucose reached a plateau (asymptotic value) between 180 to 240 min after the start of a continuous infusion of isotopically labelled glucose. All the calculations reported in the present study are based on specific activities measured between 240 and 300 min after the start of a continuous infusion of isotopically labelled glucose or after a change in exposure

temperatures.

Percent glucose oxidized

The percent of the body glucose oxidized to CO_2 was calculated from the following formula (Bergman 1963):

$$\% \text{ Glucose oxidized} = \frac{^{14}C \text{ expired (uc/min)}}{^{14}C \text{ infused (uc/min)}} \times 100 \quad ..(2)$$

Percent CO_2 from glucose

The percent of the CO_2 production derived from glucose was estimated from the asymptotic specific activities of CO_2 produced and plasma glucose as follows (Bergman 1963):

$$\% \ CO_2 \text{ from glucose} = \frac{\text{SA of } CO_2 \text{ produced (uc/mg C)}}{\text{SA of plasma glucose (uc/mg C)}} \times 100. \quad (3)$$

Glucose oxidation rate

The glucose oxidation rate was estimated from the $\% \ CO_2$ from glucose and the rate of CO_2 production (Bergman 1963):

Glucose oxidation rate = (mg/min/kg)

$$\frac{\% \ CO_2 \text{ from glucose}}{100 \times \text{Body wt (kg)}} \times \frac{\text{Rate of } CO_2 \text{ produced (mg C/min)}}{\text{mg C in 1 mg of glucose}} \quad ..(4)$$

Percent heat production from glucose

Upon complete oxidation, 1 milligram of glucose yeilds approximately 3.73 calories (West et al 1966). Therefore 3.73 times the oxidation rate of glucose gives an estimate of the rate at which calories are released during complete oxidation of glucose to CO_2 and thus the contribution of glucose oxidation to the total heat production of the animal. Hence, the percent of the heat production derived from glucose was calculated using the following formula:

$$\% \text{ Hp from glucose} = \frac{3.73 \text{ (cal/mg)} \times \text{Glucose Oxidation Rate (mg/min/kg)}}{\text{Total Hp of the animal (cal/min/kg)}} \times 100 \quad (5)$$

where Hp = Heat production

It should be noted that "any recycling will tend to lead to underestimates of the rate of entry of the tracer into the blood, and any CO_2 produced in the rumen will dilute the respired CO_2 and so tend to lead to the underestimate of the fraction of the CO_2 arising in the tissue from tracer metabolites. Nonetheless the values ..... show the relative importance of the metabolites to each other". (Ballard et al 1969).

B. Calculations of Palmitate Parameters

The isolation and separation of isotopically labelled palmitate yeilded low and unsatisfactory recoveries.

Cross contamination between samples within the gas-liquid chromatograph column was the probable cause of the unacceptable palmitate specific activity results. The use of palmitate concentration for estimating palmitate utilization was therefore considered.

In vivo studies have shown the uptake of FFA by liver to be proportional to the plasma FFA concentrations (Fine and Williams 1960; and McElroy et al 1960). Furthermore the oxidation of palmitate in vitro by skeletal muscle, diaphram and liver was found to be proportional to the concentration of palmitate in the medium (Fritz et al 1958; and Eaton and Steinberg 1961). Likewise the increase in plasma FFA concentration due to adrenaline infusion or starvation is associated with higher rates of FFA oxidation, whereas the decrease in plasma FFA concentrations due to the infusion of glucose is associated with decreased rates of FFA oxidation (see review by Steinberg 1963).

Armstrong et al (1961) measured the flux of FFA in dogs by continuous infusions of ^{14}C-palmitate, under many varying conditions which brought about variations of plasma FFA concentrations over a 30 fold range. These conditions included unanesthetized dogs in a postabsorptive state, pentobarbital anesthesia, insulin infusion, growth hormone infusion, norepinephrine infusion and sodium oleate infusion. They concluded that "under a variety of conditions changes in FFA concentrations are brought about

by changes in FFA production rate and that changes in FFA uptake are simple mass action effects of changes in FFA concentration". Therefore Armstrong et al (1961) suggested that FFA turnover could be evaluated under a variety of conditions simply by measuring FFA concentration. Experiments with sheep, carried out by West and Annison (1964), support the conclusion of Armstrong et al (1961) however they suggest that other factors are also involved in the control and uptake of FFA. They did find a linear relationship between the oxidation rate of palmitate and the plasma palmitate concentration.

Since the plasma palmitate concentrations and the isotope infusion rates in the present study are known, it was possible to estimate specific activities from the following equation:

$$\text{SA plasma palmitate (uc/mg)} = \frac{\text{Infusion rate (uc/min)}}{2.9416Y + 0.2824\ \text{(mg/min/kg)}} \times \frac{1}{\text{Body wt (kg)}} \quad \ldots\ldots (6)$$

where y = concentration of palmitate (m.mole/l)

This equation was derived by substituting the regression equation of West and Annison (1964) into the general equation for palmitate flux as follows:

General equation (Armstrong et al 1961)

Palmitate Flux (mg/min/kg) =

$$\frac{\text{Infusion rate (uc/min)}}{\text{SA plasma palmitate (uc/mg)}} \times \frac{1}{\text{Body wt (kg)}} \quad \ldots(7)$$

Regression equation (West and Annison 1964)

$$\text{Palmitate Flux (mg/min/kg)} = 2.9416Y + 0.2824 \quad \ldots\ldots(8)$$

Therefore $2.9416Y + 0.2824 =$

$$\frac{\text{Infusion rate (uc/min)}}{\text{SA plasma palmitate (uc/mg)}} \times \frac{1}{\text{Body wt (kg)}} \quad \ldots\ldots(9)$$

The rearrangement of equation 9 will give equation 6. Equation 6 may not give a precise estimate of specific activity, however if the metabolism of palmitate in the present sheep is directly proportional to their plasma palmitate concentration as the literature suggests (West and Annison 1964), then use of the derived specific activity for showing relative changes in fat metabolism should be valid (Armstrong et al 1961).

The formulas used to calculate palmitate parameters were similar to those used to calculate glucose parameters. The palmitate parameters calculated were as follows:

* Palmitate flux ..... (7)
* % CO_2 from palmitate ..... (10)

% Palmitate oxidized ..... (11)

* Oxidation rate ..... (12)

* % Heat production from palmitate .... (13)

Percent CO_2 from palmitate

The percent of the carbon dioxide derived from palmitate was calculated from the specific activity of plasma palmitate, calculated as mentioned above, and the specific activity of expired CO_2 measured during the palmitate trials:

$$\% \ CO_2 \text{ from palmitate} = \frac{\text{SA of } CO_2 \text{ produced (uc/mg C)}}{\text{SA of plasma palmitate (uc/mg C)}} \times 100 \quad .... \quad (10)$$

Percent palmitate oxidized

The percent of the palmitate flux that is oxidized may be calculated from the ratio of ^{14}C expiration to the rate of ^{14}C infused times 100:

$$\% \text{ Palmitate oxidized} = \frac{^{14}C \text{ expired (uc/min)}}{^{14}C \text{ infused (uc/min)}} \times 100 \quad .. \quad (11)$$

* Depend on the regression equation of West and Annison (1964).

Palmitate Oxidation Rate

Using the % CO_2 from palmitate, calculated previously, and the data on rate of carbon dioxide expiration (Appendix C19-C33), the oxidation rate of palmitate was calculated:

Palmitate Oxidation rate = (umole/min/kg)

$$\frac{\%\ CO_2 \text{ from palmitate}}{100 \times \text{Body wt (kg)}} \times \frac{\text{Rate of } CO_2 \text{ produced (mg C/min)}}{\text{mg C in 1 umole of palmitate}} \quad \ldots (12)$$

Percent heat production from palmitate

One micromole of palmitate is known to contain 2.398 calories of energy (West et al 1966) and therefore the percent of the heat production derived from palmitate may be calculated using a similar formula to that used for glucose:

% Hp from palmitate =

$$\frac{2.398 \text{ (cal/umole)} \times \text{Palmitate Oxidation rate (umole/min/kg)}}{\text{Rate of Hp (cal/min/kg)}} \times 100 \quad \ldots (13)$$

C. Summary of Derived Results

a) Glucose Fluxes

The glucose flux (Table 9) of fed cold conditioned sheep chronically exposed to -2°C was more than double that

Table 9. Fluxes of Plasma Glucose (mg/min/kg) and Palmitate (umole/min/kg) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose flux			Palmitate flux		
		18	-2	-24	18	-2	-24
Fed	Warm conditioned (18°C)	[a] 1.24 ±0.10		1.56 ±0.05	1.59 ±0.05		3.43 ±0.43
	Cold conditioned (-2°C)	2.19 ±0.47	3.13 ±0.35	3.10 ±0.59	1.68 ±0.03	2.30 ±0.01	2.83 ±0.30
Fasted	Warm conditioned (18°C)	1.13 ±0.05		1.53 ±0.14	2.38 ±0.07		4.99 ±0.52
	Cold conditioned (-2°C)	1.80 ±0.17	1.89 ±0.10	1.69 ±0.10	3.36 ±0.05	2.84 ±0.53	3.18 ±0.07

Comparisons		Glucose flux	Palmitate flux
I Warm vs. cold conditioned at conditioning temp.	Fed	*	*
	Fasted	*	-
II Warm vs. cold conditioned at 18°C	Fed	-	-
	Fasted	*	*
III Warm conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	-	-
V Warm vs. cold conditioned at -24°C	Fed	-	-
	Fasted	-	-

[a] Mean ± standard error of the mean

* Sign. diff. ($P < 0.05$)

of fed warm conditioned sheep at 18°C (3.1 vs 1.2 mg/min/kg). Lower rates were observed in both cold and warm conditioned animals when they were fasted (1.9 and 1.1 mg/min/kg respectively).

The glucose fluxes of both fed and fasted cold conditioned sheep exposed to 18°C were greater than those of respective warm conditioned sheep exposed to 18°C.

Warm conditioned sheep exposed to acute cold had only slightly higher glucose fluxes than those observed at 18°C (approx. 1.5 vs 1.2 mg/min/kg) regardless of whether they were fed or fasted. The glucose flux of fed cold conditioned sheep increased from 2.2 to 3.1 mg/min/kg during acute cold exposure, which was significantly higher than that observed for the warm conditioned sheep exposed to acute cold. The fasting of cold conditioned sheep resulted in glucose fluxes during acute cold (1.6 mg/min/kg) which tended towards those observed for the warm conditioned animals.

In brief. Chronic cold exposure increased glucose flux. The cold conditioning of sheep appearred to increase the glucose flux both during acute warm and acute cold exposure. Acute cold caused the glucose flux to increase in all sheep except the fasted cold conditioned sheep. Fasting did not appear to have an effect on glucose flux in warm conditioned sheep however fasting tended to inhibit the increases in glucose flux which were observed in fed cold conditioned sheep.

b) Palmitate fluxes

During chronic exposures, the cold conditioned sheep had higher palmitate flux rates (Table 9) than the warm conditioned, sheep. Fasting caused the rates to increase in both groups of sheep.

The palmitate flux of fed warm conditioned sheep was 1.6 compared to 1.7 umole/min/kg for fed cold conditioned sheep at 18°C. Fasting increased the rates of palmitate flux to 2.4 and 3.4 umole/min/kg for warm and cold conditioned sheep at 18°C, respectively.

The palmitate flux in warm conditioned animals during acute cold was more than double that observed at 18°C. The palmitate flux of fed cold conditioned animals increased from 1.7 to 2.8 umole/min/kg as the temperature changed from 18 to -24°C, however when fasted there was a slight decrease in flux from 3.3 to 3.2 umole/min/kg. Warm conditioned sheep exposed to acute cold had greater palmitate flux than cold conditioned animals in both fed and fasted states.

In brief. Both chronic and acute cold increased the palmitate flux. Cold conditioning sheep tended to increase the palmitate flux observed at 18°C but lower those at -24°C. Fasting increased palmitate flux in all cases.

c) Glucose oxidation rates

The glucose oxidation rates are summarized in Table 10.

Table 10. Oxidation Rates of Plasma Glucose (mg/min/kg) and Palmitate (umole/min/kg) of Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose oxidation			Palmitate oxidation		
		18	-2	-24	18	-2	-24
Fed	Warm conditioned (18°C)	[a]0.23 ±0.00		1.15 ±0.10	0.36 ±0.08		2.06 ±0.20
	Cold conditioned (-2°C)	0.54 ±0.12	0.72 ±0.05	1.97 ±0.25	0.55 ±0.78	0.70 ±0.02	1.57 ±0.26
Fasted	Warm conditioned (18°C)	0.20 ±0.02		0.98 ±0.10	0.85 ±0.15		3.20 ±0.19
	Cold conditioned (-2°C)	0.29 ±0.03	0.39 ±0.02	0.59 ±0.03	1.24 ±0.03	1.28 ±0.26	2.02 ±0.08

Comparisons		Glucose oxid.	Palmitate oxid.
I Warm vs. cold conditioned at conditioning temp.	Fed	*	*
	Fasted	*	-
II Warm vs. cold conditioned at 18°C	Fed	*	-
	Fasted	-	-
III Warm conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
V Warm vs. cold conditioned at -24°C	Fed	-	-
	Fasted	*	*

[a] Mean ± standard error of the mean

* Sign. diff. ($P < 0.05$)

A faster rate of glucose oxidation was observed during chronic exposure to -2°C than 18°C. Fasting had very little effect on the rate of glucose oxidation of the warm conditioned sheep at 18°C however at -2°C fasting reduced the rate from 0.72 to 0.39 mg/min/kg.

The fed cold conditioned sheep exposed to 18°C had a much higher rate of glucose oxidation than the fed or fasted warm conditioned sheep at 18°C. Fasting tended to reduce the rate of glucose oxidation in cold conditioned animals towards that observed for warm conditioned animals.

The glucose oxidation rates were significantly ($P < 0.05$) greater in all cases during acute cold exposure than at 18°C. The cold conditioned animals had the highest oxidation rates in the warm (0.54 mg/min/kg) and showed the greatest increase (to 1.9 mg/min/kg) during the acute cold period. The same animals fasted showed a much smaller increase in glucose oxidation as the temperature was changed from 18 to -24°C (0.29 to 0.50 mg/min/kg). Thus fasted cold conditioned sheep at -24°C had the lowest rates of glucose oxidation. The oxidation rates for both fed and fasted warm conditioned sheep were between those observed for fed and fasted cold conditioned sheep.

In brief. Both chronic and acute cold increased the rates of glucose oxidation. Cold conditioning of sheep increased the rate of glucose oxidation observed except in fasted sheep exposed to acute cold (-24°C). Fasting caused a

reduction in the rates of glucose oxidation in cold conditioned sheep but had little effect on the rates in warm conditioned sheep.

d) Palmitate oxidation rate

The oxidation of palmitate was greater in cold conditioned sheep at -2°C than in warm conditioned sheep at 18°C in both fed and fasted states. Fasting increased the oxidation rate of palmitate in both groups of sheep.

The cold conditioned sheep at 18°C had higher oxidation rates than the warm conditioned sheep at 18°C in both fed and fasted states. The rate of palmitate oxidation for fasted animals was more than double that observed for the fed animals at 18°C.

Acute cold exposure caused the oxidation of palmitate to increase in all cases. Warm conditioned sheep had higher rates of palmitate oxidation than cold conditioned sheep during acute cold exposure. The oxidation rates of palmitate for fasted animals were greater than those for fed animals during acute cold.

In brief. Chronic cold, acute cold and fasting increased the oxidation rate of palmitate. Cold conditioning increased the rate of oxidation observed during the warm but decreased that observed during acute cold.

e) Percent glucose oxidized to CO_2

The percent of the body glucose oxidized to CO_2 (Table 11) by sheep chronically exposed to warm or cold did not differ significantly in either fed or fasted states.

The fed cold conditioned sheep exposed to 18°C oxidized 26% of the glucose to CO_2 compared to 19% for the fed warm conditioned at 18°C. Fasting decreased the percent glucose oxidized to CO_2 to 15% in the cold conditioned sheep but had little effect on the warm conditioned sheep.

The percent of the glucose oxidized to CO_2 was increased in all cases when the sheep were exposed to acute cold. The warm conditioned sheep oxidized a greater percent of their glucose to CO_2 than the cold conditioned sheep. Fasting tended to decrease the percent oxidized to CO_2.

In brief. Chronic cold had little effect on the percent of the glucose oxidized to CO_2 whereas acute cold increased the percent 2-3 times. Cold conditioning generally decreased the percent of glucose oxidized to CO_2 except when the sheep were acutely exposed to 18°C in a fed state.

f) Percent palmitate oxidized to CO_2

The percent of the palmitate flux oxidized to CO_2 (Table 11) was significantly greater ($P < 0.05$) in fed sheep chronically exposed to -2 than 18°C. Fasting increased

Table 11. Percent of the Glucose and Palmitate Oxidized to Carbon Dioxide by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose trials			Palmitate trials		
		18	-2	-24	18	-2	-24
Fed	Warm conditioned (18°C)	[a] 18.8 ±0.8		74.0 ±6.0	17.0 ±2.0		59.3 ±1.2
	Cold conditioned (-2°C)	26.1 ±2.2	23.3 ±1.5	54.6 ±8.1	24.4 ±1.5	27.2 ±1.0	54.8 ±4.4
Fasted	Warm conditioned (18°C)	18.6 ±2.1		63.9 ±3.5	34.6 ±6.1		63.7 ±3.0
	Cold conditioned (-2°C)	15.4 ±1.0	19.9 ±1.0	34.8 ±0.7	36.9 ±0.2	37.1 ±3.4	62.7 ±3.7

Comparisons		Glucose trials	Palmitate trials
I Warm vs. cold conditioned at conditioning temp.	Fed	*	*
	Fasted	-	-
II Warm vs. cold conditioned at 18°C	Fed	*	-
	Fasted	-	-
III Warm conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
V Warm vs. cold conditioned at -24°C	Fed	-	
	Fasted	*	-

[a] Mean ± standard error of the mean

* Sign. diff. ($P < 0.05$)

the percentage in both cases such that the differences between them became insignificant.

The percent of palmitate oxidized to CO_2 was greater in fed cold conditioned sheep than fed warm conditioned sheep at 18°C. Both warm and cold conditioned sheep had increased rates of palmitate oxidation when fasted and exposed to 18°C, however the difference between them was not significant.

Acute cold increases the percent palmitate oxidized to CO_2 in all cases. During acute cold, the differences between warm and cold conditioned were not observed to be statistically significant. The percent of palmitate oxidized to CO_2 was greater for fasted than fed sheep.

In brief. Chronic cold, acute cold and fasting increased the percent of the plasma palmitate flux oxidized to CO_2. The differences between warm and cold conditioned sheep were not significant when the sheep were in a fasted state. Cold conditioning increased the percent palmitate oxidized to CO_2 at 18°C in fed sheep.

g) Percent CO_2 from glucose

The percent of the CO_2 derived from glucose (Table 12) during chronic warm exposure to 18°C was 4.1

Table 12. Percent of the CO_2 Produced from Glucose and Palmitate by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep	Glucose trials			Palmitate trials		
	18	-2	-24	18	-2	-24
Fed Warm conditioned (18°C)	[a]4.1 ±0.4		10.1 ±0.3	3.6 ±0.9		10.6 ±0.7
Cold conditioned (-2°C)	7.4 ±1.3	10.3 ±1.2	16.9 ±0.8	3.5 ±0.2	4.8 ±0.6	8.4 ±1.4
Fasted Warm conditioned (18°C)	5.1 ±0.6		10.2 ±1.1	9.7 ±0.15		16.4 ±1.0
Cold conditioned (-2°C)	5.6 ±0.7	5.6 ±0.6	6.4 ±0.5	9.8 ±0.3	9.2 ±1.2	9.4 ±0.1

Comparisons		Glucose trials	Palmitate trials
I Warm vs. cold conditioned at conditioning temp.	Fed	*	-
	Fasted	-	-
II Warm vs. cold conditioned at 18°C	Fed	-	-
	Fasted	-	-
III Warm conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	*	*
IV Cold conditioned at 18 vs. -24°C	Fed	*	*
	Fasted	-	-
V Warm vs. cold conditioned at -24°C	Fed	*	-
	Fasted	-	*

[a] Mean ± standard error of the mean

* Sign. diff. ($P<0.05$)

and 5.1% in fed and fasted sheep respectively. Sheep chronically exposed to -2°C derived a greater proportion of their CO_2 from glucose when fed (10.3%) however, not much more than the warm conditioned sheep when fasted (5.6%).

The cold conditioned sheep derived more CO_2 from glucose than the warm conditioned sheep at 18°C when fed, however when fasted the difference was negligible.

The percent CO_2 from glucose increased in all cases when the sheep were exposed to acute cold. The warm conditioned sheep derived approximately 10.1% of their CO_2 from glucose during acute cold exposure in both fed and fasted states. Fasting the cold conditioned sheep resulted in a large decrease (from 16.9% for the fed to 6.4% for the fasted) in the amount of CO_2 derived from glucose.

In brief. Both acute and chronic cold increased the percent of the CO_2 derived from glucose. The fed cold conditioned sheep were observed to derive a greater proportion of their CO_2 from glucose than the warm conditioned sheep. Fasting decreased the percent CO_2 from glucose in cold conditioned sheep but had little effect on the percent CO_2 from glucose in the warm conditioned sheep.

h) Percent CO_2 from palmitate

The differences in the percent CO_2 derived from palmitate during chronic exposures of sheep to cold or

warm were not significant in either fed or fasted states. Fasting more than doubled the percent CO_2 derived from palmitate.

Cold conditioning had little effect on the percent CO_2 derived from palmitate at 18°C.

The exposure of sheep to acute cold increased the percent CO_2 from palmitate in all cases except in the fasted cold conditioned sheep. The percent of the CO_2 derived from palmitate during acute cold exposure was greatest for the fasted warm conditioned sheep. The differences between fed warm conditioned and fed or fasted cold conditioned sheep during acute cold exposure were not significant.

In brief. Chronic cold did not alter the percent CO_2 from palmitate significantly. Similarly cold conditioning had little effect during acute warm exposure, however during acute cold exposure cold conditioning appeared to reduce the percent CO_2 derived from palmitate. Both acute cold and fasting increased the percent CO_2 derived from palmitate in most cases.

i) Percent heat production from glucose

The fed chronically cold exposed sheep derived a significantly greater ($P<0.05$) percent of their heat production (Table 13) from glucose than did the fed

Table 13. Percent of the Heat Production Produced from Glucose and Palmitate by Sheep Conditioned to 18 or -2°C and Exposed for Measurement to 18, -2 and -24°C

Sheep		Glucose trials			Palmitate trials		
		18	-2	-24	18	-2	-24
Fed	Warm conditioned (18°C)	[a]3.4 ±0.4		7.8 ±0.5	3.7 ±0.8		10.9 ±1.1
	Cold conditioned (-2°C)	5.9 ±1.1	7.8 ±0.8	13.1 ±0.7	3.6 ±0.1	4.8 ±0.6	8.0 ±1.2
Fasted	Warm conditioned (18°C)	3.5 ±0.2		7.3 ±0.8	9.7 ±0.4		14.4 ±0.6
	Cold conditioned (-2°C)	4.2 ±0.5	3.4 ±0.3	4.4 ±0.5	9.8 ±0.0	8.4 ±1.1	8.9 ±0.1

Comparisons		Glucose trials	Palmitate trials
I	Warm vs. cold conditioned at conditioning temp.	Fed	*	-
		Fasted	-	-
II	Warm vs. cold conditioned at 18°C	Fed	-	-
		Fasted	-	-
III	Warm conditioned at 18 vs. -24°C	Fed	*	*
		Fasted	*	*
IV	Cold conditioned at 18 vs. -24°C	Fed	*	*
		Fasted	-	*
V	Warm vs. cold conditioned at -24°C	Fed	*	-
		Fasted	*	*

[a] Mean ± standard error of the mean

* Sign. diff. ($P < 0.05$)

chronically warm exposed sheep (7.8 compared to 3.4% respectively). Fasted animals derived approximately 3.4% of their heat production from glucose during both chronic warm and chronic cold exposures.

The cold conditioned animals derived a slightly higher percent of their heat production from glucose at 18°C than did the warm conditioned sheep in both fed and fasted states.

Acute cold caused significant increases ($P < 0.05$) in the contribution of glucose to heat production in all cases with the exception of fasted cold conditioned sheep. The percent of the total heat production derived from glucose during acute cold exposure was greatest in the fed cold conditioned animals and least in the fasted cold conditioned animals. Fasting had little effect on the warm conditioned animals exposed to acute cold.

In brief. Fed sheep when exposed to acute or chronic cold derived a greater percent of their heat production from glucose than when exposed to warm. Cold conditioning caused an increase in the contribution of glucose to heat production at both 18 and -24°C when the sheep were fed. Fasting decreased the contribution of glucose to heat production in cold conditioned sheep.

j) Percent heat production from palmitate

The contribution of palmitate to total heat production was greater in fasted than fed sheep.

The percent of the heat production from palmitate in cold conditioned sheep exposed to 18 or -2°C was not significantly different from that of the warm conditioned sheep exposed to 18°C.

The percent of the heat production from palmitate increased in most cases when the sheep were exposed to acute cold with the exception of the fasted cold conditioned sheep. The warm conditioned sheep derived a greater proportion of their calories from palmitate during acute cold than the cold conditioned sheep.

SUMMARY OF RESULTS

The five comparisons made for each parameter are summarized in Tables 14 (Comparison I, II and III) and 15 (Comparisons IV and V). Control values are presented for each comparison and the values to be compared have been expressed in terms of the percent change from the control.

Comparison I was made to see the effect of chronic cold on the parameters measured. Therefore the cold conditioned sheep at their conditioning temperature (-2°C) were compared to the controls - warm conditioned sheep at their conditioning temperature (18°C). Chronic cold was observed to increase both glucose and palmitate catabolism in fed sheep to meet the demands of the increased heat production. The percent increase in glucose parameters was greater than that observed for palmitate parameters. The glucose flux and oxidation increased significantly when fasted sheep were exposed to chronic cold whereas the palmitate parameters changed only slightly. There was little change in the heat production derived from either substrate when the sheep were in a fasted state.

Comparison II was made to see the effect of cold conditioning sheep on the parameters measured when there was no cold stress imposed. Therefore the cold conditioned sheep acutely exposed to 18°C were compared to the controls - warm conditioned sheep at their conditioning

Table 14. Summary of Comparisons I, II and III of the Results. In each comparison the results from the warm conditioned sheep exposed to 18°C have been used as controls. The differences between the controls and the alternate treatments have been expressed as percents of the controls.

Parameter	Controls [b]WC at 18°C	Comp. I [a]CC at -2°C (%)	Comp. II CC at 18°C (%)	Comp. III WC at -24°C (%)
Fed				
Hematocrits (%)	30.0	14*	8*	14*
RQ	0.79	-6	-3	-4
Hp (cal/min/kg)	24.5	44*	25*	103*
Gluc. Conc. (mg/100 ml)	59.1	26	18	24
Palm. Conc. (umole/ml)	0.109	146*	17	376*
Gluc. Flux (mg/min/kg)	1.24	152*	77	26*
Palm. Flux (umole/min/kg)	1.59	45*	6	116*
Gluc. Oxid. (mg/min/kg)	0.23	213*	135*	400*
Palm. Oxid. (umole/min/kg)	0.36	94*	53	472*
% Gluc. to CO_2	18.8	24*	39*	294*
% Palm. to CO_2	17.0	60	43	249*
% CO_2 from Gluc.	4.1	151*	80	146*
% CO_2 from Palm.	3.6	32	-3	192*
% Hp from Gluc.	3.4	126*	71	127*
% Hp from Palm.	3.7	28	-3	193*
Fasted				
Hematocrits (%)	32.0	-4	15	9*
RQ	0.72	-10*	3	-4
Hp (cal/min/kg)	21.5	80*	30*	136*
Gluc. Conc. (mg/100 ml)	68.8	19	15	-13
Palm. Conc. (umole/ml)	0.285	36	77*	205*
Gluc. Flux (mg/min/kg)	1.13	67*	59*	35*
Palm. Flux (umole/min/kg)	2.38	19	41*	110*
Gluc. Oxid. (mg/min/kg)	0.20	95*	45	390*
Palm. Oxid. (umole/min/kg)	0.85	51	46	277*
% Gluc. to CO_2	18.6	7	-17	244*
% Palm. to CO_2	32.6	7	7	84*
% CO_2 from Gluc.	5.1	10	10	100*
% CO_2 from Palm.	9.7	-6	7	69*
% Hp from Gluc.	3.5	-3	25	111*
% Hp from Palm.	9.7	-13	17	48*

* Significantly different from control ($P<0.05$)

[a] Cold Conditioned [b] Warm Conditioned

Table 15. Summary of Comparisons IV and V of the Results.

In Comparisons IV the results from the cold conditioned sheep exposed to 18°C have been used as the controls (IV) and in Comparisons V the results from the warm conditioned sheep exposed to -24°C have been used as the controls (V). The differences between the controls and the alternate treatments have been expressed as percents of the controls.

Parameter	Controls (IV) [a]CC at 18°C	Comp. (IV) CC at -24°C (%)	Controls (V) [b]WC at -24°C	Comp. (V) CC at -24°C (%)
Fed				
Hematocrits (%)	32.5	11*	34.2	5
RQ	0.77	-5	0.76	-4
Hp (cal/min/kg)	30.7	68*	49.8	4
Gluc. Conc. (mg/100ml)	69.7	0	73.6	-5
Palm. Conc. (umole/ml)	0.128	202*	5.19	-25
Gluc. Flux (mg/min/kg)	2.19	42*	1.56	99*
Palm. Flux (umole/min/kg)	1.68	68*	3.43	-17
Gluc. Oxid. (mg/min/kg)	0.54	265*	1.15	71*
Palm. Oxid. (umole/min/kg)	0.55	184*	2.06	-24
% Gluc. to CO_2	26.1	109*	74.0	-26
% Palm. to CO_2	24.4	125*	59.3	-8
% CO_2 from Gluc.	7.4	128*	10.1	67*
% CO_2 from Palm.	3.5	141*	10.6	-21
% Hp from Gluc.	5.9	124*	7.8	69*
% Hp from Palm.	3.6	122*	10.9	-27
Fasted				
Hematocrits (%)	36.7	8*	35.0	14
RQ	0.74	-7*	0.69	0
Hp (cal/min/kg)	28.0	84*	50.8	1
Gluc. Conc. (mg/100ml)	79.3	-2	59.9	30
Palm. Conc. (umole/ml)	0.504	-8	8.7	-46
Gluc. Flux (mg/min/kg)	1.80	-6	1.53	10
Palm. Flux (umole/min/kg)	3.36	-5	4.99	-93
Gluc. Oxid. (mg/min/kg)	0.29	103*	0.98	-40*
Palm. Oxid. (umole/min/kg)	1.24	63*	3.20	-37*
% Gluc. to CO_2	15.4	126*	63.9	-46*
% Palm. to CO_2	36.9	70*	63.7	-2
% CO_2 from Gluc.	5.6	14	10.2	-37
% CO_2 from Palm.	9.8	-4	16.4	-43*
% Hp from Gluc.	4.2	5	7.3	-40
% Hp from Palm.	9.8	-9*	14.4	-38*

* Significantly different from control ($P<0.05$)

[a] Cold Conditioned [b] Warm Conditioned

temperature (18°C). Most parameters measured were slightly higher in the cold conditioned animals than the warm conditioned animals at 18°C in both fed and fasted states. Glucose metabolism was generally higher in the fed cold conditioned sheep than the fed warm conditioned sheep, with a significant difference ($P < 0.05$) in the rate of glucose oxidation. The percent increase in heat production from glucose was greater than that observed for palmitate in fed sheep.

Comparison III was made to see the effect of acute cold on warm conditioned sheep. Therefore the warm conditioned sheep exposed to -24°C were compared to the controls - same sheep at conditioning temperature (18°C). Glucose and palmitate metabolism were greatly increased to meet the increased demand of heat production with acute cold in both fed and fasted sheep. The percent increase in palmitate parameters with acute cold was slightly higher than the percent increase in glucose parameters in fed sheep mainly due to a lower relative increase in glucose flux. In fasted sheep, the percent change in the glucose parameters were greater than those of palmitate.

Comparison IV was made to see the effect of acute cold on cold conditioned sheep. Therefore the cold conditioned sheep exposed to -24°C were compared to the controls - same sheep at 18°C. Acute cold caused significant

increases ($P<0.05$) in most of the parameters measured when sheep were in a fed state. The relative increases in the contribution of glucose and palmitate to heat production were similar in fed sheep. In the fasted sheep exposed to acute cold, there was very little change in glucose or palmitate concentration or flux although the rates of oxidation were increased significantly ($P<0.05$). There was little change in the proportion of the heat production derived from glucose or palmitate when animals were fasted.

Comparison V was made to see the effect of cold conditioning on sheep when exposed to acute cold. Therefore the cold conditioned sheep at -24°C were compared to the controls - warm conditioned sheep at -24°C. Most of the glucose parameters measured were significantly higher in fed cold conditioned sheep than fed warm conditioned sheep exposed to -24°C whereas, both glucose and palmitate parameters were generally lower in fasted cold conditioned sheep than fasted warm conditioned sheep.

GENERAL DISCUSSION

The loss of weight observed in the cold conditioned sheep during the experimental period may have been due to ketosis. The combination of fasting and cold temperatures could have overtaxed the metabolic pathways resulting in increased ketone production. Bergman (1973) stated that appetite is usually depressed or absent in animals showing clinical signs of ketosis. The death of one of the cold conditioned sheep shortly after she had been fasted was also probably due to ketosis.

The restlessness observed in the sheep probably increased the variability of the results; however, differences were still apparent between treatments.

The hematocrits of the experimental sheep were observed to increase when the sheep were exposed to acute cold. Slee found a similar effect on hematocrits when sheep were exposed to acute cold (see Halliday et al 1969). The sheep chronically exposed to cold also tended to have higher hematocrits than those maintained in the warm. Slee did not find any differences in hematocrits of sheep chronically exposed to 8°C and 30°C (see Halliday et al 1969). The higher hematocrits observed in the chronically cold exposed sheep reported herein may be due to the lower temperature of -2°C to which they were exposed. Cold conditioned sheep had higher hematocrits at 18°C than did

warm conditioned sheep. The changes in hematocrits observed in the present sheep in response to cold may have been initially illicited by stress. Cold is a stress factor which increases an animal's demand for energy. Stress causes spleen contraction which releases red blood cells and hence causes a larger hematocrit and blood volume (Guyton 1971). Guyton (1971) postulates that the increased blood volume insures a rapid return of blood to the heart and the greater number of circulating red blood cells gives the blood a greater oxygen carrying capacity. The higher hematocrits observed in the present study were associated with higher heat productions and hence may have been necessary to supply the increased oxygen demand.

The heat production was found to be greater in both chronic and acute cold exposed sheep than in warm exposed sheep which was expected since Blaxter (1962) calculated the lower critical temperature of sheep with 5 mm fleeces to be 31°C when fasted, 25°C when given a maintenance ration and 18°C when full fed. Fed sheep had higher heat production than fasted sheep probably due to the energy expenditure required to digest the feed. However, when exposed to acute cold the differences between fed and fasted sheep disappeared. Blaxter (1958), Joyce and Blaxter (1964) suggest that the nutritional level of an animal has no effect on total heat production below the animals's critical temperature. Therefore differences in heat

production between fed and fasted sheep would probably not be expected at an acute cold temperature of -24°C. Cold conditioned sheep had higher heat productions than warm conditioned sheep at 18°C in both fed and fasted states which may have been due to an increased resting metabolic rate of cold conditioned sheep. Other researchers (Slee 1972; and Webster et al 1969) also suggested that exposure to cold increased the resting metabolic rate of sheep. Although the previous studies may be confounded by differences in feed intake, Young (1972) and Young and Christopherson (1974) also found elevated resting metabolic rates in cattle on constant levels of feeding.

The purpose of this thesis was to determine the extent to which glucose and fat in sheep contribute to the increased heat production associated with cold. It was on the basis of protein corrected RQ's that Graham et al (1959) suggested that below the critical temperature the source of additional heat for sheep was entirely increased fat catabolism. A similar suggestion was made for cattle (Blaxter and Wainman 1961). The RQ's presented in Table 4 were not corrected for protein catabolism and as well they were calculated for relatively short periods of time (15 min). The RQ's were decreased by chronic cold exposure, especially in fasted animals. Acute cold and fasting caused significant decreases in the RQ's compared to warm exposed or fed animals respectively. These results

are therefore in agreement with Graham et al (1959) in that they indicate that fat is being utilized to a greater extent in cold exposed sheep. However, this does not necessarily mean that the use of other specific substrates is curtailed. The recycling of substrates in vivo may not be apparent from RQ's. Recently it has been suggested that both liver gluconeogenesis and muscle glycolysis increase with cold exposure of rats (Nakagawa and Nagai 1971). A similar phenomenon could exist in sheep. If liver gluconeogenesis and muscle glycolysis increase in sheep exposed to cold such that there is no depletion in body glucose stores, then the changes in glucose metabolism would not affect RQ's. Similarly an increase in the Cori cycle would have no direct effect on RQ's since it is an anaerobic cycle which does not produce CO_2. Leng (1970) suggested that the recycling of glucose carbon might be an important process when gluconeogenic substrates are in short supply or when the demand for gluconeogenesis is substantial.

There were no statistically significant differences observed in plasma glucose concentration due to thermal conditioning or exposure temperature. It is generally recognized that control mechanisms in animals tend to maintain a relatively constant blood glucose concentration (Krebs 1964). However, the plasma glucose concentrations of sheep chronically exposed to cold were observed to be slightly higher than those chronically exposed to warm

temperatures. This is in agreement with the results of Halliday et al (1969) who found that glucose levels were higher in shorn sheep chronically exposed to 8 than to 30°C. Olsen and Trenkle (1973) found that cattle also had slightly higher plasma glucose concentrations when exposed to prolonged cold. Halliday et al (1969) exposed shorn sheep to an acute cold of -25°C and observed the glucose concentrations to approximately double. The glucose concentrations of fed warm conditioned sheep were also observed to increase (from 59.7 to 73.6 mg/100 ml) upon exposure to acute cold (-24°C) in the present studies, however not nearly to the extent observed by Halliday et al (1969). Acute cold exposure appeared either to have little effect or to cause slight decreases in plasma glucose concentration of cold conditioned and/or fasted sheep. Similarly, Pernod et al (1972) found that glucose concentration remained fairly constant in dogs exposed to acute cold of -25°C.

The fasting of dogs for 7 to 11 days was observed to have no significant effect on glucose concentration compared to normal dogs (Steele et al 1968). In the present study, the glucose concentrations were observed to be generally higher in fasted than fed sheep with the exception of warm conditioned sheep exposed to acute cold. Cold conditioned sheep also tended to have higher glucose concentrations than warm conditioned sheep except when they were exposed to acute cold in a fed state.

Although the differences in glucose concentrations between treatments were not statistically significant, the changes in specific activity of glucose and expired CO_2 during the glucose trials indicate that there were significant changes in the utilization of glucose with cold exposure. "The rate of glucose uptake is well known not to be related to glucose concentration in any simple way because a variety of agents and hormones influence the readiness with which glucose is taken up at any given plasma glucose concentration." (Armstrong et al 1961).

In order to better understand the changes in glucose metabolism with cold a number of calculations were made (see derived results, page 59) using the specific activities of plasma glucose and CO_2 produced as the basis. The results of these calculations have been summarized in Tables 9-13.

It may be seen from Table 9 that the glucose flux was 3.13 mg/min/kg in fed sheep chronically exposed to cold compared to 1.24 mg/min/kg in fed sheep chronically exposed to warm. The glucose fluxes of the fed sheep chronically exposed to warm were low compared to those commonly reported in the literature (see review by Leng 1970). This may be related to the larger size of sheep used in the present study compared to other studies (Leng 1970) or to some inapparent effect of experimental condition. The rate of glucose oxidation was also greater in the chronically cold

exposed sheep compared to the warm exposed sheep (Table 10), with the net effect that a greater proportion of the expired CO_2 was derived from glucose carbon during chronic cold exposure (10.3% compared to 4.1% respectively). If this is expressed in terms of the percent of the total heat production coming from glucose then approximately 7.7% of the calories used for heat production during chronic cold exposure came from glucose compared to 3.4% during chronic warm exposure. The glucose flux and oxidation rate were both much lower in the chronically cold exposed sheep when they were fasted than when they were fed whereas, fasting had little effect on these parameters in the chronically warm exposed sheep. In ruminants, gluconeogenesis is usually reduced by fasting (Annison and White 1961; Kronfeld and Simesen 1961; Leng 1970 and Bergman 1973). Bergman (1963) found that the average turnover rate of glucose (glucose flux) was 1.39 mg/min/kg in fed sheep compared to only 0.95 mg/min/kg in fasted sheep. However his sheep were fasted 3-6 days compared to only 36 h in the present studies. It is possible that the warm conditioned sheep after 36 hours fasting are still obtaining substantial amounts of substrate from the gut, whereas the cold conditioned sheep would utilize these substrates at a much higher rate due to their higher heat production and therefore would become dependent on body stores much sooner. If this is the case, then fasting for 36 h would be expected to have a greater effect on cold conditioned than warm conditioned sheep as seen in this study. The net effect was that approximately

3.4% of the heat production was derived from glucose in both the chronically cold and warm exposed sheep when they were fasted. However, the rate of heat production was greater in the fasted chronically cold exposed sheep compared to the fasted chronically warm exposed sheep and hence a greater amount of heat is being produced from glucose in the chronically cold exposed sheep.

The estimated glucose flux for fed cold conditioned sheep exposed to 18°C was 2.4 mg/min/kg compared to 1.24 mg/min/kg for warm conditioned sheep exposed to 18°C. Similarly, the glucose oxidation rates were 0.54 and 0.23 mg/min/kg, respectively. The greater glucose flux and oxidation rate observed in the cold conditioned sheep compared to the warm condition sheep resulted in a greater proportion of the total heat production being derived from glucose in the cold conditioned sheep (5.9% compared to 3.4%). Fasting the cold conditioned animals caused a slight decline in glucose flux at 18°C from 2.19 to 1.80 mg/min/kg and a marked decline in the rate of oxidation from 0.54 to 0.29 mg/min/kg. This means that the percent of the glucose flux oxidized to CO_2 in the cold conditioned sheep exposed to 18°C was reduced from 26.1% to 15.4% by fasting, which is even less than the 18.6% observed for the fed or fasted warm conditioned sheep at 18°C.

The exposure of the experimental sheep to acute cold (-24°C) resulted in higher glucose flux and oxidation

rates than observed for the same sheep at 18°C, with the exception of the fasted cold conditioned sheep and hence a greater percent of the total heat production was derived from glucose in most cases (see Table 13). Pernod et al (1972) found the plasma glucose turnover (glucose flux) and oxidation rates also increased in dogs when they were exposed to acute cold (-25°C) however there was a drop in the glucose participation to CO_2 from 19 to 12%. In the present study the level of glucose flux and oxidation attained varied depending on whether the animals were warm or cold conditioned; fed or fasted. The fed cold conditioned animals were observed to have much higher glucose flux (3.10 mg/min/kg) and oxidation rates (1.97 mg/min/kg) than all other treatments during acute cold. However, a greater proportion of the glucose flux was oxidized to CO_2 in the warm conditioned sheep compared to the cold conditioned sheep in both fed and fasted states (Table 11). In all cases the proportion of glucose oxidized to CO_2 increased with acute cold exposure. Pernod et al (1972) also noted that a greater proportion of the glucose in dogs was quickly oxidized to CO_2 when the dogs were exposed to acute cold. Both the glucose flux and the glucose oxidation rate were reduced in the cold conditioned sheep acutely exposed to cold when fasted. This is most likely due to the lower rate of gluconeogenesis in fasted compared to fed ruminants (Annison and White 1961; Kronfeld and Simesen 1961; Leng 1970; and Bergman 1973). The percent of the glucose flux

oxidized to CO_2 during acute cold exposure was lowest for the fasted cold conditioned sheep. The net result was that the fed cold conditioned sheep derived a greater proportion of their heat production from glucose during acute cold than either fed or fasted warm conditioned sheep. However, when the cold conditioned sheep were fasted they derived less heat production from glucose than the warm conditioned sheep in either a fed or fasted state.

It is therefore concluded that both chronic and acute cold exposure increase the contribution of glucose to the total heat production in sheep. Cold conditioning appears to increase utilization of glucose for heat production in fed sheep although the percent of the glucose flux being oxidized to CO_2 is slightly less than in warm conditioned sheep. Fasting appears to have little effect on the utilization of glucose in warm conditioned sheep however, it causes marked reductions in both flux and oxidation in cold conditioned sheep.

The results indicate that there might be a slight inhibition of glucose oxidation in fed cold conditioned sheep and when fasted this inhibition increases markedly. The fact that the highest glucose concentrations were observed in the cold conditioned fasted sheep (Table 6) indicate that glucose oxidation was not limited by availability. An increased release of growth hormone might explain the inhibition of glucose oxidation observed. Fasting and/or stress stimulate the release of growth hormone (Grodsky 1971). Machlin et al (1968) observed an

increase in growth hormone in young anesthetized sheep during induction of hypothermia with ice packs. Olsen and Trenkle (1973) also observed an increase in plasma growth hormone levels of cattle exposed to prolonged cold. The cow which appeared the most stressed by cold in their studies had the greatest increase in growth hormone. Growth hormone, in times of increased energy demand, may spare protein and carbohydrate at the expense of fat since growth hormone stimulates overall protein synthesis, (Grodsky 1971) impairs glucose utilization (Hollobaugh et al 1968) and increases fat utilization (Knobil and Hotchkiss 1964).

Ruminants in a physiological emergency may be placed in a carbohydrate insufficiency and develop ketosis (Patterson and Cunningham 1969). The slow response or lack of response of ketogenesis in ruminants to glucose therapy emphasizes that it is not glucose availability but rather its utilization which is impaired in ketosis (Patterson and Cunningham 1969), possibly by the hypersecretion of growth hormone (Seekles 1948; Kronfeld 1963; 1965; and Patterson and Cunningham 1969). A similar interpretation may be placed on results obtained in the present study. However, hypoglycemia which is commonly associated with ketosis (see review by Patterson and Cunningham 1969) and, which has been suggested to be the stimulus for the secretion of growth hormone (Roth et al 1963; and Knobil 1964) was not observed in the present study. Growth hormone can cause hyperglycemia (Williams 1962) and therefore the relatively high levels of glucose observed in the fasted

cold conditioned sheep may have resulted after an increase in the plasma level of growth hormone. The lowered appetite observed after fasting the cold conditioned sheep may be due to the production of ketones which is known to impair appetite (Bergman 1973).

In this, as in similar studies, it has been assumed that the metabolism of palmitate is representative of the metabolism of the total FFA fraction. The plasma palmitate concentrations were increased by chronic cold exposure, acute cold exposure and fasting. Halliday et al (1969) and Slee and Halliday (1968) found that exposure of sheep to chronic and acute cold increased the plasma levels of free fatty acids, which is in agreement with the results presented herein. Similarly Bost and Dorleac (1965) found that plasma FFA increased as environmental temperature fell. Patterson (1963) also noted an increase in FFA in ewes that was apparently due to adverse weather conditions whereas Karihaloo et al (1970) did not observe any effect of wintering ewes at low temperatures on plasma FFA. The FFA concentration in cattle was observed to increase when they were exposed to an acute cold of -20^{o}C (Thompson et al 1972). Armstrong et al (1961); Issekutz et al (1964) and West and Annison (1964) suggest that the FFA concentration is dependent on the rate of mobilization and that the amount of FFA catabolized is controlled by the circulating level according to the law of mass action. Therefore it is evident from the changes in palmitate concentration observed when sheep were exposed to

cold, that fat is an important metabolic substrate for the increased heat production associated with cold.

Estimates of the contribution of palmitate to heat production have been made on the basis of palmitate concentration and the specific activity of CO_2 during palmitate trials (see page 62 of Derived Results). The palmitate flux in fed chronically cold exposed sheep was approximately 2.3 umoles/min/kg compared to 1.59 umoles/min/kg for fed chronically warm exposed sheep. The rate of palmitate oxidation was 0.70 umoles/min/kg for the fed chronically cold exposed sheep and 0.36 umoles/min/kg for the fed chronically warm exposed sheep. The net result was that fed chronically cold exposed sheep derived a slightly greater percent of their calories from palmitate than did the fed chronically warm exposed sheep (4.78 vs. 3.73%). The fluxes and oxidation rates of palmitate were higher in fasted sheep than fed sheep when chronically exposed to either warm or cold which resulted in a higher proportion of the calories being derived from palmitate in fasted sheep than fed sheep.

The cold conditioned sheep exposed to 18°C had slightly higher palmitate fluxes and palmitate oxidation rates than the warm conditioned sheep exposed to 18°C. However, with the higher total heat production observed in the cold conditioned sheep at 18°C the net result was that

there was no difference in the percent of the heat production derived from palmitate between the two groups.

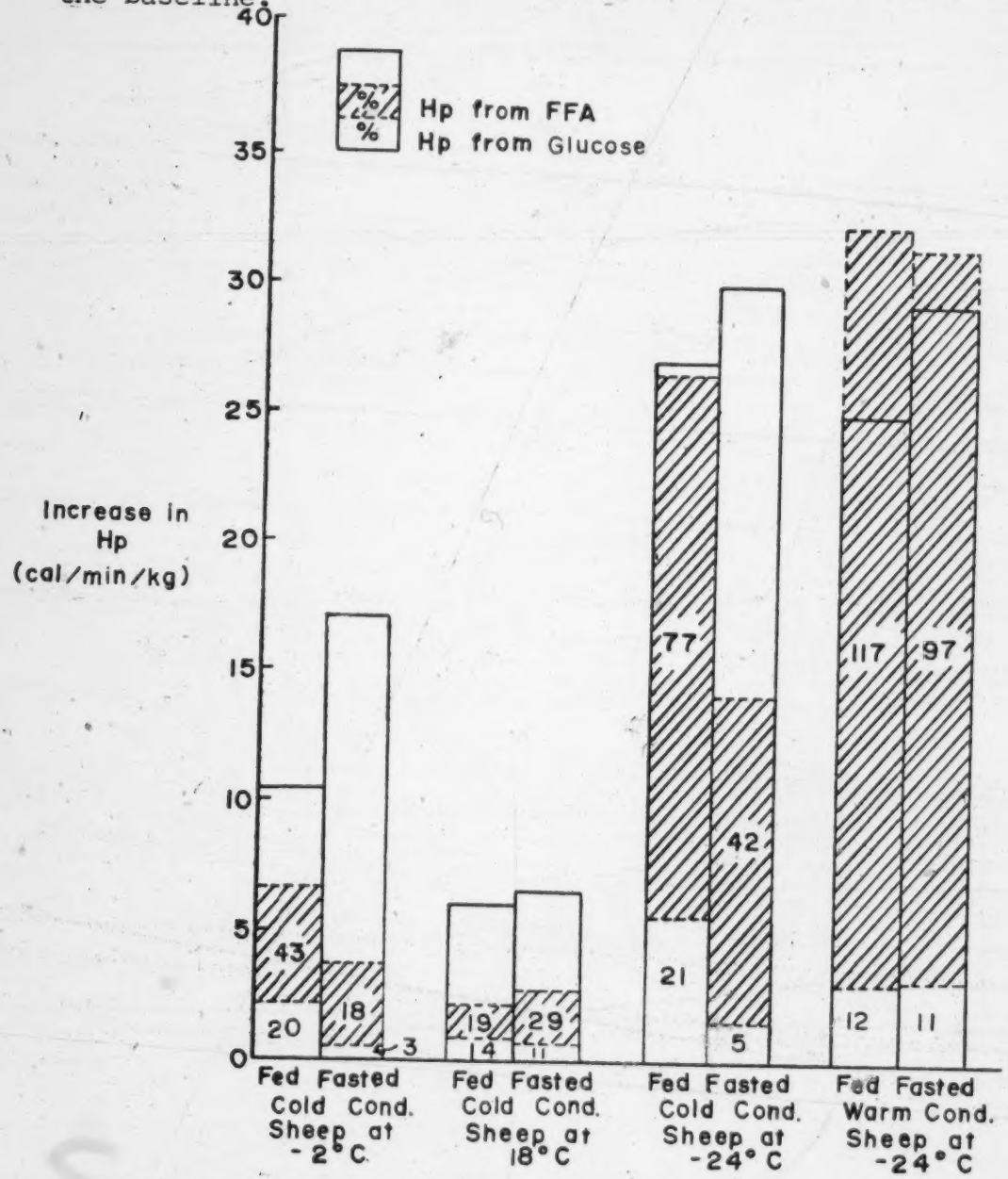
The palmitate flux and oxidation rates were greater during acute cold exposure than at 18°C, with the exception of the palmitate flux of fasted cold conditioned sheep. A greater proportion of the palmitate flux was quickly oxidized to CO_2 during acute cold exposure than at 18°C. Vincent-Falquet et al (1972) and Paul and Holmes (1973) found that dogs exposed to acute cold also showed higher palmitate turnover (flux) and oxidation rates and that a greater proportion of the palmitate was quickly oxidized to CO_2 than in the warm. Both the flux and the oxidation rates of palmitate were higher in warm conditioned sheep than cold conditioned sheep in both fed and fasted states during acute cold exposure. There was no difference in the percent of the palmitate flux oxidized to CO_2 between warm and cold conditioned sheep. The net result was that warm conditioned sheep derived a greater percent of their calories for heat production from palmitate than cold conditioned sheep in both fed and fasted states.

Therefore it may be concluded that the use of palmitate for heat production is increased by acute cold, chronic cold and fasting. Cold conditioning of sheep appears to slightly reduce the proportion of the total heat production derived from palmitate.

As mentioned previously, the purpose of this thesis is to determine the relative contributions of glucose and FFA in sheep to the increased heat production associated with cold. The following figure (Figure 5) illustrates the contribution of FFA and glucose to the increased heat production associated with cold. The increase in heat production due to cold was estimated by determining the differences in heat production between the warm conditioned sheep at 18°C and the other treatments for both fed and fasted sheep. The increased heat production derived from glucose and palmitate with cold were estimated similarly. The increased heat production from palmitate was converted to increased heat production from FFA by dividing by 0.16. The factor 0.16 was used since palmitate made up approximately 16% of the total FFA in the experimental sheep (see Appendix D).

Approximately 20% of the increased heat production in sheep chronically exposed to -2°C can be accounted for by the oxidation of glucose compared to approximately 43% by the oxidation of FFA. Fasting reduces the contribution of glucose to approximately 3.2% and of FFA to approximately 18% of the increased heat production. The reduction in the glucose contribution to the increased heat production is most likely related to the lower rate of gluconeogenesis in fasted sheep. In the fed animal the gut most likely contributes a significant proportion of the substrates required for heat production, however, in fasted animals

Figure 5. The Percent of the Increased Heat Production (Hp) Associated with Cold Exposure of Sheep Accounted for by Increases in Glucose and Free Fatty Acid (FFA) Oxidation. *Measurements from the warm conditioned sheep at their conditioning temperature (18°C) were used to establish the baseline.



* Warm Cond. at 18°C	Total Hp (cal/min/kg)	% Hp from Glucose	% Hp from FFA
Fed	24.6	3.4	23.1
Fasted	21.5	3.5	60.6

the contribution of substrates from the gut is most likely reduced and hence the animal becomes more dependent on body stores. Therefore the reason for the decrease in calories contributed by FFA to the increased heat production in the fasted chronically cold exposed sheep is not readily apparent unless the body fat depots were depleted to such an extent that the sheep became more dependent on body protein when fasted. It is possible that the decrease was associated with incomplete combustion of FFA and that the sheep may have been in a ketotic state.

The fed cold conditioned sheep acutely exposed to 18°C derived approximately 14% of their calories for the increased heat production from glucose and 19% from FFA. Fasting resulted in a slightly smaller percent of the increased heat production coming from glucose (11%) and a larger percent coming from FFA (29%).

Approximately 21% of the increased heat production of fed cold conditioned sheep acutely exposed to -24°C can be accounted for by glucose and approximately 77% by FFA. Fasting these animals resulted in significant decreases in the contribution of glucose and FFA to the increased heat production associated with acute cold exposure. It is probable that this response to fasting is similar to that previously described for the cold conditioned sheep when exposed to chronic cold. The fact that the fasted cold conditioned sheep had the highest glucose concentration but the lowest glucose oxidation rates during acute cold exposure would indicate that glucose metabolism was inhibited.

Similarly, the reduction in palmitate utilization may indicate some impairment of FFA utilization.

The percent of the increased heat production contributed by FFA upon exposure of warm conditioned sheep to an acute cold temperature of -24°C was estimated to be 117% when sheep were fed and 97% when fasted. These results support Graham et al's (1959) and Blaxter and Wainman's (1961) conclusion that in ruminants fat alone can account for the increased heat production associated with cold. However, the results presented herein also indicate that glucose accounts for approximately 11% of the increased heat production associated with acute cold exposure. The energy supplied by the gut probably decreases during cold exposure, at least in the fasted animals and hence the sheep must rely more on body stores for normal maintenance energy. It is therefore probably inaccurate to suggest that the increase in fat metabolism is solely directed at the increase in heat production since it is likely that more of the maintenance requirements are now being met by the mobilization of body stores. The results presented herein indicate that both FFA and glucose contribute to heat production in warm conditioned sheep exposed to acute cold.

The cold conditioned sheep appeared to utilize glucose to a much greater extent than the warm conditioned sheep which is evident from the higher percent of the heat

production derived from glucose during both the chronic and acute cold exposures of these sheep. However, both glucose and palmitate metabolism appeared to be impaired in these sheep when fasted whereas fasting appeared to have very little effect on either glucose or palmitate metabolism in warm conditioned sheep.

The source of energy for the increased heat production not accounted for by glucose or FFA catabolism can only be speculated at the present time. The logical alternate source of energy is protein however as mentioned previously the maintenance of body protein is crucial to an animal's survival. Therefore it is tempting to suggest that nitrogen recycling increases concurrently with heat production, although there is no evidence of this to date. An increase in the amount of ammonia from catabolized protein reincorporated into amino acids in the rumen by microorganisms and then reabsorbed, would conserve protein mass at times of increased protein catabolism. However, the role of protein in cold induced thermogenesis is not clear due to the limited research in this area to date.

This study showed that glucose as well as fat oxidation increase to meet the increased requirements for heat production when sheep are exposed to cold. Further research is required to determine the mechanism whereby glucose oxidation was impaired in cold conditioned sheep keeping in mind the possible role of growth hormone. Such

research might increase our understanding of common stress disorders in which a carbohydrate deficiency is implicated in the etiology, such as ketosis and pregnancy toxemia, both of which are enhanced by adverse weather conditions.

The limitations of the present study should be realized if the results are to be interpreted fairly. Only three animals were used per sample. Sheep were used as the experimental animal with the assumption that they were representative of ruminants. These sheep were exposed to controlled environments within the laboratory which may have yeilded different results than would have been found under natural conditions. Estimates of FFA utilization were based mainly on plasma palmitate concentrations. In the light of the present research it would seem advisable to increase the concentration of gluconeogenic precursors, possibly by increasing grain supplement, in times of adverse weather conditions. However further research on the effects of cold on substrate utilization is required before we can assess its effect on production and hence make unqualified recommendations for feed and maintenance.

SUMMARY AND CONCLUSIONS

The effects of acute and chronic cold on carbohydrate and fat metabolism in ruminants were studied. Six closely clipped mature ewes were used in both fed and fasted states. The sheep were either warm or cold conditioned by maintaining them at 18 or -2°C respectively. Measurements were taken at the respective conditioning temperatures and when both groups of sheep were acutely exposed to -24°C. Additional measurements were taken when the cold conditioned sheep were acutely exposed to 18°C. Isotopically labelled palmitate or glucose was infused into the sheep throughout each measurement period in order to monitor glucose or palmitate metabolism. The analysis of labelled palmitate from plasma was not satisfactory and therefore the estimates of palmitate utilization were based on the plasma palmitate concentrations and the specific activity of CO_2 produced.

The hematocrits and metabolic rates were found to be higher and the respiratory quotients lower in most cases during cold exposures (-2 and -24°C) compared to warm exposures (18°C).

Chronic cold exposed sheep in a fed state were found to have an average glucose flux of 3.13 mg/min/kg, glucose oxidation of 0.72 mg/min/kg, palmitate flux of 2.30 mmoles/min/kg and palmitate oxidation of 0.70 mmole/min/kg

compared to 1.24 mg/min/kg, 0.23 mg/min/kg, 1.59 mmole/min/kg and 0.36 mmole/min/kg respectively for chronically warm exposed sheep. The percent of the heat production derived during chronic cold from glucose was 125% and from palmitate 28% higher than that observed in the chronically warm exposed sheep. In fasted sheep the response of glucose and palmitate flux and oxidation to chronic cold was similar but smaller than that for fed sheep. There was little change in the proportion of the heat production derived from either substrate due to chronic cold exposure when sheep were in a fasted state.

The cold conditioned sheep exposed to acute warm (18°C) were found to have elevated resting metabolic rates when both fed and fasted. Both glucose and palmitate oxidation were slightly higher in the cold conditioned sheep at 18°C compared to the warm conditioned sheep at 18°C.

The exposure of fed warm conditioned sheep to acute cold resulted in significant increases in glucose and palmitate flux. The oxidation rates of the two substrates were increased by more than 400% by exposure to acute cold and hence the proportion of the heat production derived from the two substrates measured increased significantly. Fasting lowered the response observed when warm conditioned sheep were exposed to acute cold however, the changes in both glucose and palmitate metabolism were significant.

Fed cold conditioned sheep exposed to acute cold were also found to have increased fluxes and oxidation rates of both palmitate and glucose however, the relative increases were not as great as those observed for fed warm conditioned sheep. In the fasted animals there was little change in the flux of either palmitate or glucose although the rate of oxidation of these substrates was increased when exposed to acute cold.

Cold conditioned sheep exposed to acute cold ($-24^{\circ}C$) had higher rates of glucose flux and oxidation and lower rates of palmitate flux and oxidation than warm conditioned sheep exposed to acute cold when in a fed state, however when in a fasted state all these parameters were lower for the cold conditioned sheep.

The contribution of FFA and glucose to heat production in sheep is increased by exposure to both acute and chronic cold whereas fasting increases the FFA contribution but decreases the contribution of glucose. Cold conditioning increases the sheeps capacity for glucose utilization, however a glucose sparing mechanism also comes into play and becomes very active in the fasted animal.

It is now apparent that glucose as well as FFA contribute to cold thermogenesis in ruminants.

BIBLIOGRAPHY

Annison, E.F. 1960. Plasma non-esterified fatty acids in sheep. Aust. J. Agr. Res. 11:58.

Annison, E.F., K.J. Hill and D. Lewis. 1957. Studies on the portal blood of sheep. 2. Absorption of volatile fatty acids from the rumen of the sheep. Biochem. J. 66:592.

Annison, E.F., R.A. Leng, D.B. Lindsay and R.R. White. 1963. The metabolism of acetic acid, propionic acid and butyric acid in sheep. Biochem. J. 88:248.

Annison, E.F. and D.B. Lindsay. 1961. Acetate utilization in sheep. Biochem. J. 78:777.

Annison, E.F. and J.L. Linzell. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over-all metabolism and to milk formation. J. Physiol. 175:372.

Annison, E.F. and R.R. White. 1961. Glucose utilization in sheep. Biochem. J. 80:162.

Armstrong, D.T., R. Steele, N. Altszuler, A. Dunn, J.J. Bishop and R.C. DeBodo. 1961. Regulation of plasma free fatty acid turnover. Am. J. Physiol. 201:9.

Astwood, E.B. 1970. Anterior pituitary hormones and related substances. In L.S. Goodman and A. Gilman, The Pharmacological Basis of Therapeutics. 4th ed. Collier-Macmillan Canada Ltd., Toronto. p. 1512.

Baker, N., R.A. Shipley, R.E. Clark, G.E. Incefy. 1959. C^{14} studies in carbohydrate metabolism; glucose pool size and rate of turnover in the normal rat. Am. J. Physiol. 196:245.

Ballard, F.J., R.W. Hanson and D.S. Kronfeld. 1969. Gluconeogenesis and lipogenesis in tissue from ruminant and nonruminant animals. Fed. Proc. 28:218.

Bartley, J.C. and A.L. Black. 1966. Effect of exogenous glucose on glucose metabolism in dairy cows. J. Nutr. 89:317.

Baxter, C.F., M. Kleiber and A.L. Black. 1955. Glucose metabolism in the lactating cow. Biochem. Biophys. Acta. 17:354.

Bergman, E.N. 1963. Quantitative aspects of glucose metabolism in pregnant and nonpregnant sheep. Am. J. Physiol. 204:147.

Bergman, E.N. 1968. Glycerol turnover in the non-pregnant and ketotic pregnant sheep. Am. J. Physiol. 215:865.

Bergman, E.N. 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Vet. 37:341.

Bergman, E.N., M.L. Katz, and C.F. Kaufman. 1970. Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. Am. J. Physiol. 219:785.

Bergman, E.N., R.S. Reid, M.G. Murray, J.M. Brockway and F.G. Whitelow. 1965. Interconversions and production of volatile fatty acids in the sheep rumen. Biochem. J. 97:53.

Bergman, E.N., D.J. Starr, and S.S. Reukin. 1968. Glycerol metabolism and gluconeogenesis in the normal and hypoglycemia ketotic sheep. Am. J. Physiol. 215:874.

Bergman, E.N. and J.E. Wolff. 1971. Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. Am. J. Physiol. 221:586.

Black, A.L., M. Kleiber and A.M. Brown. 1961. Butyrate metabolism in the lactating cow. J. Biol. Chem. 236:2399.

Blaxter, K.L. 1958. Nutrition and climatic stress in farm animals. Proc. Nutr. Soc. 17:191.

Blaxter, K.L. 1962. In the Energy Metabolism of Ruminants. Hutchinson and Co. Ltd., London.

Blaxter, K.L. and F.W. Wainman. 1961. Environmental temperature and the energy metabolism and heat emission of steers. J. Agr. Sci. 56:81.

Bost, J. and E. Dorleac. 1965. Action du Froid sur le taux plasmatique des acides gras non esterifies (FFA) chez le mouton. Comptes rendus des seances de la Societe de Biologie 159;2209.

Bowden, D.M. 1971. Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: a review. Can. J. Anim. Sci. 51:1.

Brengelmann, G. and A.C. Brown. 1965. Temperature regulation. In T.C. Ruch and H.D. Patton, Physiology and Biophysics. 19th ed., W.B. Saunders Co., Philadelphia. p. 1050.

Cahill, G.F. (Jr.), E.B. Marliss and T.T. Aoki. 1970. Fat and nitrogen metabolism in fasting man. In B. Jeanrenaud and D. Hepp, Hormone and Metabolic Research Adipose Tissue - Regulation and Metabolic Functions. Academic Press, New York. p. 181.

Calvert, D.T., D.P. Clough, J.D. Findlay and G.E. Thomson. 1972. Hypothalamic cooling, heat production and plasma lipids in the ox. Life Sci. 11.223.

Carlson, L.D. 1963. Temperature regulation and cold acclimation. Physiologist 6:29.

Crampton, E.W. and L.E. Lloyd. 1959. Fundamentals of Nutrition. W.H. Freeman and Co., San Francisco.

Depocas, F. 1962. Body glucose as a fuel in white rats exposed to cold: results with fasted rats. Am. J. Physiol. 202:1015.

Depocas, F., and R. Masironi. 1960. Body glucose as fuel for thermogenesis in the white rat exposed to cold. Am. J. Physiol. 199:1051.

Dole, V.P. and H. Meinertz. 1960. Microdeterminations of long-chain fatty acids in plasma and tissues. J. Biol. Chem. 235:2595.

Eaton, P. and D. Steinberg. 1961. Effects of medium chain fatty acid concentration, epinephrine and glucose on palmitate 1-C^{14} oxidation and incorporation into neutral lipids by skeletal muscle in vitro. J. Lipid Res. 2:376.

Eldsen, S.R. and A.T. Phillipson. 1948. Ruminant digestion. Ann. Rev. Biochem. 17:705.

Evans, C.L. 1956. The temperature and heat-balance of the body. In Principles of Human Physiology, 12th ed., J. and A. Churchill Ltd., London. p. 1056.

Fine, M.B. and R.H. Williams. 1960. Effect of fasting, epinphrine and glucose and insulin on hepatic uptake of nonesterified fatty acids. Am. J. Physiol. 199:403.

Ford, E.J.H. 1963. Glucose utilization in pregnant sheep. Biochem. J. 88:427.

Ford, E.J.H. 1965. The importance of glucose in ruminant metabolism. European Assoc. An. Prod. Publ. 11:21.

Ford, E.J.H. and P.E.B. Reilly. 1969. The utilization of plasma free amino acid and glucose carbon by sheep. Res. Vet. Sci. 10:409.

Fritz, I.B., D.G. Davis, R.H. Holtrop and H. Dundee. 1958. Fatty acid oxidation by skeletal muscle during rest and activity. Am. J. Physiol. 194:379.

Graham, N. McC., F.W. Wainman, K.L. Blaxter and D.G. Armstrong. 1959. Environmental temperature, energy metabolism and heat regulation in sheep. 1. Energy metabolism in closely clipped sheep. J. Agr. Sci. 52:13.

Grodsky, G.M. 1971. The chemistry and functions of the hormones. In H.A. Harper, Review of Physiological Chemistry. Lange Medical Publication, Los Altos, California. p. 413.

Guyton, A.C. 1971. In Textbook of Medical Physiology. W.B. Saunders Co., Toronto. p. 372.

Halliday, R., A.R. Sykes, J. Slee, A.C. Field and A.J.F. Russel. 1969. Cold exposure of Southdown and Welsh mountain sheep. 4. Changes in concentrations of free fatty acids, glucose, acetone, protein-bound iodine, protein and antibody in the blood. Anim. Prod. 11:479.

Hammarstrand, K., J.M. Juntunen and A.R. Hennes. 1969. A method of collection of gas-liquid chromatographic effluent with cigarette filtertips. Anal. Biochem. 27:172.

Hart, J.S. 1957. Climate and temperature-induced changes in the energetics of homeotherms. Rev. Can. Biol. 16:133.

Hartmann, P.E. and A.K. Lascelles. 1965. Variation in the concentration of lipids and some other constituents in the blood plasma of cows at various stages of lactation. Aust. J. Biol. Sci. 18:114.

Heald, P.J. 1951. Assessment of glucose containing substances in rumen micro-organisms during a digestive cycle in sheep. Brit. J. Nutr. 5:84.

Héroux, O. 1963. Patterns of morphological, physiological and endocrinological adjustments under different environmental conditions of cold. Fed. Proc. 22:789.

Hird, F.J.R. and M.J. Weidemann. 1964. Transport and metabolism of butyrate by isolated rumen epithelium. Biochem. J. 92:585.

Ho, R.J. 1970. Dependence of hormone stimulated lipolysis on ATP and cyclic AMP levels in fat cells. In B. Jeanrenaud and D. Hepp, Hormone and Metabolic Research Adipose Tissue - Regulation and Metabolic Functions. Academic Press, New York. p. 83.

Ho, R.J., R. England, R.A. Johnson and H.C. Meng. 1970. Dependence of hormone- stimulated lipolysis on energy metabolism. Am. J. Physiol. 219:125.

Hollobaugh, S.L., M. Tzagournis, R.L. Folk, F.A. Kruger and G.J. Hamwi. 1968. The diabetogenic action of human growth hormone: glucose - fatty acid interrelationships. Metabolism 17:485.

Issekutz, B., H.I. Miller and I.K. Rodah. 1963. Effect of exercise on FFA metabolism of pancreatectomized dogs. Am. J. Physiol. 205:645.

Jarrett, I.G. and B.J. Potter. 1957. The metabolism of lower fatty acids in diabetic sheep. Aust. J. exp. Biol. 35:103.

Jones, G.B. 1965. Determination of the specific activity of labelled blood glucose by liquid scintillation using glucose pentaacetate. Anal. Biochem. 12:249.

Joyce, J.P. and K.L. Blaxter. 1964. Respiration in sheep in cold environments. Res. Vet. Sci. 5:506.

Karihaloo, A.K., A.J.F. Webster and W. Combs. 1970. Effects of cold, acute starvation and pregnancy on some indices of energy metabolism in Lincoln and Southdown sheep. Can. J. An. Sci. 50:191.

Kayser, C. 1937. Variations du quotient respiratoire en fonction de la temperature du milieu chez le rat, le pigeon et le cobaye. Compt. Rend. Soc. Biol. 126:1219.

Knobil, E. and J. Hotchkiss. 1964. Growth Hormone. Ann. Rev. Physiol. 26:47.

Krebs, H.A. 1964. Gluconeogenesis. Proc. Roy. Soc. B. 159:545.

Kronfeld, D.S. 1963. Ruminant ketosis: a speculative approach. Ann. N.Y. Acad. Sci. 104:799.

Kronfeld, D.S. 1965. Growth hormone - induced ketosis in the cow. J. Dairy Sci. 48:342.

Kronfeld, D.S. 1965. Plasma non-esterified fatty acid concentration in the dairy cow: Response to nutritional and hormonal stimuli, and significance in ketosis. Vet. Rec. 77:30.

Kronfeld, D.S. and M.G. Simesen. 1961. Glucose biokinetics in sheep. Am. J. Physiol. 201:639.

Leng, R.A. 1970. Glucose synthesis in ruminants. Advances in Vet. Med. Comp. Med. 14:209.

Leng, R.A. and E.F. Annison. 1963. Metabolism of acetate, propionate and butyrate by sheep - liver slices. Biochem. J. 86:319.

Leng, R.A., J.W. Steel and J.R. Luick. 1967. Contribution of propionate to glucose synthesis in sheep. Biochem. J. 103:785.

Leng, R.A. and C.E. West. 1969. Contribution of acetate, butyrate, palmitate, stearate and oleate to ketone body synthesis in sheep. Res. Vet. Sci. 10:57.

Lindsay, D.B. 1961. Endocrine control of metabolism in ruminants. In Digestive Physiology and Nutrition of the Ruminant; Proceedings. London, Butterworths p. 235.

Lindsay, D.B. 1970. Carbohydrate metabolism in ruminants. In A.T. Phillipson, Physiology of Digestion and Metabolism in the Ruminant. Oriel Press, Newcastle upon Tyne, England. p. 438.

Lindsay, D.B. 1971. Changes in the pattern of glucose metabolism in growth, pregnancy and lactation in ruminants. Proc. Nutr. Soc. 30:272.

Machlin, L.J., K. Takahasi, M. Herino, F. Hertelendy, R.S. Gordon and Kipnis. 1968. Regulation of growth hormone secretion in non-primate species. In A. Pecile and E.E. Muller, Growth Hormone. Excerpta Medica Foundation, Amsterdam, The Netherlands. p. 292.

Masoro, E.J. 1966. Effect of cold on metabolic use of lipids. Physiol. Rev. 45:67.

Masoro, E.J., A.I. Cohen and S.S. Panagos. 1954. Effect of exposure to cold on some aspects of hepatic acetate utilization. Am. J. Physiol. 179:451.

Masoro, E.J. and S.S. Panagos. 1956. Effects of short chain fatty acids on hepatic acetate metabolism in cold exposure and fasting. Proc. Soc. Exptl. Biol. Med. 93:277.

McClymont, G.S. and B.P. Setchell. 1956. Non-utilization of acetate and utilization of glucose by the brain of sheep. Aust. J. Biol. Sci. 9:184.

McElroy, W.J., W.L. Siefert and J.J. Spitzer. 1960. Relationship of hepatic uptake of free fatty acids to plasma concentration. Proc. Soc. Exp. Biol. Med. 104:20.

McLean, J.A. 1970. Simultaneous direct and indirect calorimetric measurements on cattle. European Assoc. An. Prod. Publ. 13:229.

Menahan, L.A., L.H. Shultz and W.G. Hoekstra. 1966. Factors affecting ketogenesis from butyric acid in the ruminant. J. Dairy Sci. 49:835.

Milligan, L.P. 1971. Energetic efficiency and metabolic transformations. Fed. Proc. 30:1454.

Nakagawa, H. and K. Nagai. 1971. Cold adaptation. 1. Effect of cold exposure on gluconeogenesis. Am. J. Physiol. 69:923.

NRC. 1970. Nutrient requirements of beet cattle. National Academy of Sciences, Washington.

Olsen, J.D. and A. Trenkle. 1973. Exposure of cattle to controlled subzero temperature: growth hormone, glucose and free fatty acid concentrations in plasma. Am. J. Vet. Res. 34:747.

Owen, O.E., A.P. Morgan, H.G. Kemp, J.M. Sullivan, M.G. Herrera and G.F. Cahill. 1967. Brain metabolism during fasting. J. Clin. Invest. 46:1589.

Pagé, E. and L. Chénier. 1953. Effects of diet and cold environment on the respiratory quotient of the white rat. Rev. Can. Biol. 12:530.

Palmquist, D.L. 1972. Palmitic acid as a source of endogenous acetate and B-hydroxybutyrate in fed and fasted ruminants. J. Nutr. 102:1401.

Patterson, D.S.P. 1963. Some observations on the estimations of non-esterified fatty acid concentrations in cow and sheep plasma. Res. Vet. Sci. 4:230.

Patterson, D.S.P., K.N. Burns, N.F. Cunningham, C.N. Herbert and N. Saba. 1964. Plasma concentrations of glucose and non-esterified fatty acids (N.E.F.A.) in the pregnant and lactating ewe and the effect of dietary restriction. J. Agr. Sci. Camb. 62:253.

Patterson, D.S.P. and N.F. Cunningham. 1969. Nutritional disorders of ruminants. Metabolic and hormonal aspects of bovine ketosis and pregnancy toxaemia in the ewe. Proc. Nutr. Soc. 28:171.

Paul, P. and W.L. Holmes. 1973. Free fatty acid metabolism during stress: exercise, acute cold exposure and anaphylactic shock. Lipids. 8:142.

Pennington, R.J. 1952. The metabolism of short-chain fatty acids in sheep. 1. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. Biochem. J. 51:251.

Pernod, A., J.C. Vincent-Falquet, M. Jomain and Y. Minaire. 1972. Glucose and glycogen as fuels for thermogenesis in cold exposed dogs. Life. Sci., 11:475.

Reid, R.L. 1968. The physiopathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxemia. Advances Vet. Sci. 12:163.

Roth, J., S.M. Glick, R.S. Yalow and S.A. Berson. 1963. Hypoglycermia: a potent stimulus to secretion of growth hormone. Sci. 140:987.

Russel, A.J.F., J.M. Doney and R.L. Reid. 1967. The use of biochemical parameters in controlling nutritional state in pregnant ewes, and the effect of under-nourishment during pregnancy on lamb birth-weight J. Agr. Sci. Camb. 68:351.

Schambye, P. 1951. Volatile acids and glucose in portal blood of sheep. Nord. Vetmed. 3:555.

Seekles, L. 1948. Gastro-intestinal autointoxication in cattle and horses. Br. vet. J. 104:238.

Seeley, R.C., D.G. Armstrong and J.C. Macrae. 1967. Feed carbohydrates - the contribution of the end-products of their digestion to energy supply in the ruminant. European Assoc. An. Prod. 12:93.

Slee, J. 1972. Habituation and acclimatization of sheep to cold following exposures of varying length of severity. J. Physiol. 227:51.

Slee, J. and R. Halliday. 1968. Some effects of cold exposure, nutrition and experimental handling on serum free fatty acid levels in sheep. Anim. Prod. 10:67.

Steele, R., B. Winkler, I. Rathgeb, C. Bjerknes and N.A. Altszuler. 1968. Plasma glucose and free fatty acid in normal and long-fasted dogs. Am. J. Physiol. 214:313.

Steinberg, D. 1963. In J.K. Grant, The Control of Lipid Metabolism. Academic Press, Inc., New York. p. 111.

Thompson, G.E. and D.P. Clough. 1972. The effect of cold exposure on plasma lipids of the new-born and adult ox. Quart. 5. Expt. Physiol. 57:192.

Vincent-Falquet, J., A. Pernod, J. Forichon and Y. Minaire. 1972. Free fatty acids as the major fuel for thermogenesis in dogs. Life Sci. 11:725.

Wakil, S.J. and R. Bressler. 1962. Fatty acid metabolism and ketone body formation. Metabolism 11:742.

Wang, C.H. and D.L. Willis. 1965. Design and execution of radio tracer experiments. Radio tracer Methodology in Biological Science. Prentice Hall, Inc., Englewood Cliffs, New Jersey. p. 224.

Webster, A.J.F. 1966. The establishment of thermal equilibrium in sheep exposed to cold environments. Res. Vet. Sci. 7:454.

Webster, A.J.F., A.M. Hicks, F.L. Hays. 1969. Cold climate and cold temperature induced changes in heat production and thermal insulation of sheep. Can. J. Physiol. Pharmacol. 47:553.

West, C.E. and E.F. Annison. 1964. Metabolism of palmitate in sheep. Biochem. J. 92:573.

West, E.S., W.R. Todd, H.S. Mason and J.T. Van Bruggen. 1966. Energy metabolism. In Textbook of Biochemistry. 4th ed. Collier-Macmillan Canada, Ltd., Toronto. p. 970.

White, R.G., J.W. Steel, R.A. Leng, and J.R. Luick. 1969. Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism. Biochem. J. 114:203.

Williams, R.H. 1962. In Disorders in Carbohydrate and Lipid Metabolism. W.B. Saunders Co., Philadelphia. p. 27.

Wolff, J.E., E.N. Bergman and H.H. Williams. 1972. Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. Am. J. Physiol. 223:438.

You, S.S., R.W. You and E.A. Sellers. 1950. Effect of thyroidectomy, adrenalectomy and burning on the urinary nitrogen excretion of the rat maintained in a cold environment. Endocrinology 47:156.

Young, B.A. 1972. Influence of adaptation to cold on resting heat production of beef cows. Proc., Western Sect., Am. Soc. Anim. Sci. 23:270.

Young, B.A. 1974. Instrumentation for animal calorimetry. Fifth West. Canadian Heat Transfer Conf., Saskatoon. p. 3.

Young, B.A. and R.J. Christopherson. 1974. Effect of prolonged cold exposure on digestion and metabolism in ruminants. International Livestock Environment Symposium. University of Nebraska. Lincoln, Nebraska.

Young, D.R. and S.F. Cook. 1955. Effect of environmental temperature and dietary protein on urinary nitrogen excretion of rats. Proc. Soc. Exp. Biol. Med. 89:482.

APPENDIX A

Feed Test Report

Two samples (#4508 and #4509) of the alfalfa pellets which made up the sheeps' ration were sent for analysis to the Soil and Feed Testing Laboratory, Alberta Department of Agriculture, Edmonton, Alberta. The "Feed Test Report" is shown in the following table:

[a]Sample #	Moisture %	Protein %	Fiber %	Calcium %	Phosphorous %	Nitrate %	Digestible Energy (Mcal/kg)
4508	6.9	17.1	33.6	1.93	.20	.22	2838
4509	6.8	17.0	32.6	1.95	.20	.17	2809

[a] all results are given on a sample basis.

APPENDIX B

Methods

		Page
B1	Isolation of Glucose as Glucose Pentaacetate for the Determination of Specific Activity	123
B2	Extraction of FFA from Plasma	125
B3	Methylation of FFA	127

APPENDIX B1

Isolation of Glucose as Glucose Pentaacetate for the Determination of Specific Activity

This procedure is based on the procedure of Jones (1965).

Reagents - Stable tungstic acid (Hycel)
Glacial acetic acid
Distilled water
Acetic anhydride
Postassium acetate
Carrier solution - 50 mg/ml glucose in 0.2% benzoic acid
Scintillation fluid
- 12 g 25-diphenyloxazole
- 0.3g p-bis [2-(5-phenyloxazolyl)] benzene
- 3l of toluene

Procedure

1) 3 ml of plasma were added to 12 ml of stable tungstic acid, shaken vigorously, let stand 20 min and centrifuged at 1000 X g for 20 min.

2) 10 ml of the supernatant were added to 3 ml of carrier solution and 5 drops of glacial acetic acid in a 50 ml beaker. The solution was then evaporated to dryness.

3) 1.5 ml of acetic anhydride and 60-70 mg potassium acetate were added to the beaker. The beaker was then covered with aluminum foil and incubated at 80-90°C for 45-60 min.

4) 15 ml of hot distilled water was added and the solution boiled for 2-3 min or until all oily globules disappeared.

5) The solution was immediately filtered through a heated filter into a centrifuge tube by vacuum, cooled and centrifuged at 10,000 X g (Temp. of cent. = 4°C)

6) The supernatant was decanted and 15 ml of hot distilled water was added to the precipitate. The precipitate was redisolved by heating in a boiling water bath and stirring.

7) The solution was cooled and centrifuged at 10,000 X g for 10 min (Temp. of cent. = 4°C.

8) Step 6 was repeated.

9) The solution was cooled and the crystals formed were collected on filter paper (Whatman #1) and washed with cold water.

10) The crystals were placed in a tared scintillation vial, dried for 1 h at 105°C and then placed in a dessicator to cool.

11) The scintillation vial with crystals was weighed and then 15 ml of toluene based scintillation fluid was added. The activity of this solution was read.

APPENDIX B2

Extraction of FFA from Plasma

This procedure is based on the procedure of Dole and Meinertz (1960).

Reagents - Dole's reagent
- 40 parts by vol. isopropyl alcohol
- 10 parts by vol. heptane
- 1 part by vol. 1N HCl

Standard
- 75 mg arachidic acid per litre of heptane

Distilled water
Heptane
n-Pentane
1N NaOH
1N HCl
Thymol blue
- 0.01% in 70% methanol

Procedure

1) 4 ml of plasma were shaken with 20 ml of Dole's reagent in 50 ml screw cap test tubes and then let stand for 5 min.

2) 6 ml of standard, 8 ml of distilled water and 6 ml of heptane were added. The tubes were shaken and then allowed to stand for 10 min.

3) The upper heptane layer was transferred to a clean test tube and 1 ml of thymol blue was added, shaken and let stand. (Yellow)

4) 1N NaOH was added a drop at a time until the solution became alkaline. (Blue)

5) The lower blue layer was transferred to a clean test tube and washed twice with 5 ml of pentane. The pentane was discarded.

6) 1N HCl was added a drop at a time until the solution became acidic (Red) and then this phase was extracted twice with 5 ml of heptane.

APPENDIX B2

Extraction of FFA from Plasma

This procedure is based on the procedure of Jones (1965).

Reagents - Dole's reagent
- 40 parts by vol. isopropyl alcohol
- 10 parts by vol. heptane
- 1 part by vol. 1N HCl

Standard
- 75 mg arachidic acid per litre of heptane

Distilled water
Heptane
n-Pentane
1N NaOH
1N HCl
Thymol blue
- 0.01% in 70% methanol

Procedure

1) 4 ml of plasma were shaken with 20 ml of Dole's reagent in 50 ml screw cap test tubes and then let stand for 5 min.

2) 6 ml of standard, 8 ml of distilled water and 6 ml of heptane were added. The tubes were shaken and then allowed to stand for 10 min.

3) The upper heptane layer was transferred to a clean test tube and 1 ml of thymol blue was added, shaken and let stand. (Yellow)

4) 1N NaOH was added a drop at a time until the solution became alkaline. (Blue)

5) The lower blue layer was transferred to a clean test tube and washed twice with 5 ml of pentane. The pentane was discarded.

6) 1N HCl was added a drop at a time until the solution became acidic (Red) and then this phase was extracted twice with 5 ml of heptane.

7) The two pentane extracts were combined in a clean tube and evaporated to dryness under nitrogen for methylation.

APPENDIX B3

Methylation of FFA

Reagents - Melthylation reagent
- 35% Boron trifluoride solution
- 10% Boron trifluoride in methanol

20% n-pentane

45% methanol

Distilled water

n-Pentane

Procedure

1) 5 ml of methylation reagent were added to the dried FFA extract under nitrogen and then the tubes were tightly sealed with screw caps.

2) The tubes were heated in a boiling water bath for 30 minutes and then cooled.

3) 5 ml of water were added and then extracted twice with 5 ml of pentane.

4) The pentane extracts were combined, sealed under nitrogen and stored at 15°C for isolation of the individual FFA.

APPENDIX C Measurements by Trial

Appendix Table	Sheep #	Nutritional Status	[a]Condition	[b]Measurement Temp. (°C)	Page
[c]Glucose trials					
C1	1080	Fed	Warm	18, -24	129
C2	1090	Fed	Warm	18, -24	130
C3	1020	Fed	Warm	18, -24	131
C4	1110	Fed	Cold	18, -24	132
C5	1330	Fed	Cold	18, -24	133
C6	1530	Fed	Cold	18, -24	134
C7	1080	Fasted	Warm	18, -24	135
C8	1090	Fasted	Warm	18, -24	136
C9	1020	Fasted	Warm	18, -24	137
C10	1110	Fasted	Cold	18, -24	138
C11	1330	Fasted	Cold	18, -24	139
C12	1530	Fasted	Cold	18, -24	140
C13	1110	Fed	Cold	-2	141
C14	1330	Fed	Cold	-2	142
C15	1530	Fed	Cold	-2	143
C16	1110	Fasted	Cold	-2	144
C17	1330	Fasted	Cold	-2	145
C18	1530	Fasted	Cold	-2	146
Palmitate trials					
C19	1080	Fed	Warm	18, -24	147
C20	1090	Fed	Warm	18, -24	148
C21	1020	Fed	Warm	18, -24	149
C22	1110	Fed	Cold	18, -24	150
C23	1330	Fed	Cold	18, -24	151
C24	1530	Fed	Cold	18, -24	152
C25	1080	Fasted	Warm	18, -24	153
C26	1090	Fasted	Warm	18, -24	154
C27	1020	Fasted	Warm	18, -24	155
C28	1110	Fasted	Cold	18, -24	156
C29	1530	Fasted	Cold	18, -24	157
C30	1110	Fed	Cold	-2	158
C31	1530	Fed	Cold	-2	159
C32	1110	Fasted	Cold	-2	160
C33	1530	Fasted	Cold	-2	161

[a] Sheep were maintained at either 18°C (warm) or -2°C (cold).

[b] Measurements taken at approx. 18, -2 and -24°C were summarized in the Results section p. 34.

[c] In appendices C1-C18, the first value listed after the heading INF (uc X 10^{-4}/min) is the rate of U-^{14}C-glucose infusion and the second value is the rate of 1-^{3}H-glucose infusion. Both radioisotopes were included in the infusates, however, measurements for 1-^{3}H-glucose were not used in the Results section since they did not appear to yeild any additional information.

APPENDIX C 1 GLUCOSE TRIAL 1 SHEEP # 1080

FED WARM WT. (KG)=83.0 INF. (UCX10-4/MIN)= 1377.5 942.2

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	19.0	334.8	282.4	29.8	41.5	45.6	30.9	1.74
270	19.0	334.8	282.4	29.8	46.2	35.8	23.9	1.74
285	19.0	328.3	269.2	29.0	43.8	37.8	23.9	1.93
300	19.0	315.3	262.7	28.9	45.6	38.0	25.3	2.10
330	-10.0	669.3	538.1	28.0	51.1	39.9	27.3	1.82
360	-20.0	550.0	458.4	30.0	51.1	47.3	32.2	1.64
390	-20.5	645.9	522.0	32.2	62.9	31.8	20.8	1.63
420	-21.5	711.2	561.1	31.2	68.9	32.2	20.0	1.79
480	-23.0	695.6	552.5	31.5	66.2	29.2	18.8	2.03
540	-25.0	832.2	643.8	33.5	70.0	31.8	20.8	2.55
570	-25.0	832.2	611.1	34.5	70.2	29.4	18.7	2.73
585	-25.0	858.2	624.1	0.0	0.0	0.0	0.0	2.67
600	-25.0	858.2	624.1	34.9	71.1	26.3	15.3	2.67
630	11.0	468.0	341.2	31.2	69.3	27.8	16.7	2.48
660	12.0	422.5	315.0	30.2	64.9	32.7	20.9	2.62

APPENDIX C 2 GLUCOSE TRIAL 2 SHEEP # 1090

FED WARM WT. (KG)=68.0 INF. (UCX10-4/MIN)= 1423.8 990.9

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL ------UCX10-4/MG	1-3H-GL	SA CO2 C------
240	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
270	18.0	455.9	376.7	30.6	64.4	36.2	29.6	1.31
285	18.0	389.9	337.0	29.0	70.2	35.7	25.3	1.28
300	18.0	383.2	330.4	29.0	67.1	38.5	28.8	1.31
330	-10.0	654.5	528.9	31.5	73.3	36.1	25.2	2.81
360	-20.0	786.7	634.6	34.0	73.3	36.3	23.7	2.09
390	-20.0	718.6	560.4	33.1	77.2	34.6	23.9	2.31
420	-21.0	718.6	573.6	33.2	76.0	35.3	24.1	2.63
480	-23.0	768.9	591.4	34.5	71.3	34.1	23.1	3.06
540	-24.0	833.5	623.6	34.4	68.9	33.2	22.1	3.14
570	-24.0	791.8	589.0	35.1	72.8	31.5	19.9	3.06
585	-24.0	804.8	602.1	35.0	68.7	31.6	21.5	3.09
600	-24.0	837.7	634.8	35.2	69.9	31.7	20.5	3.22
630	10.0	441.3	362.3	32.8	66.5	34.5	22.7	2.97
660	14.0	316.2	296.4	32.0	61.0	39.9	26.3	2.59

APPENDIX C 3 GLUCOSE TRIAL 3 SHEEP # 1020

FED WARM WT. (KG)=70.0 INF. (UCX10-4/MIN)= 1425.7 976.8

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	20.0	425.2	322.2	33.0	60.4	36.9	26.0	1.57
270	20.0	418.8	322.2	31.0	68.9	41.2	28.1	1.62
285	20.0	413.5	323.1	31.0	65.1	0.0	0.0	1.62
300	20.0	420.0	323.1	31.2	68.9	42.0	29.7	1.62
330	-10.0	1018.1	837.7	36.5	71.3	45.3	31.2	1.83
360	-17.5	812.0	625.1	36.6	74.3	40.3	27.5	1.92
390	-20.0	668.0	507.4	34.0	82.1	37.3	25.8	1.77
420	-20.0	713.1	565.2	35.5	81.3	37.8	24.9	2.23
480	-21.0	713.1	565.2	34.8	81.3	37.2	24.5	3.18
540	-22.0	676.6	568.0	35.0	81.7	35.3	23.1	3.46
570	-23.0	774.4	626.8	35.5	80.0	33.6	22.0	3.57
585	-24.0	774.4	626.8	35.5	80.7	33.5	22.1	3.66
600	-24.0	774.4	626.8	37.5	78.1	33.5	22.5	3.66
630	11.0	314.1	295.2	32.8	74.4	35.4	23.8	3.08
660	14.0	314.1	295.2	32.5	70.7	37.9	25.5	3.08

APPENDIX C 4 GLUCOSE TRIAL 4 SHEEP # 1110

FED COLD WT. (KG)=79.0 INF. (UCX10-4/MIN)= 1426.4 1002.6

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG	C------	
240	20.0	465.9	407.6	35.5	86.7	35.7	25.7	1.70
270	20.0	446.5	388.2	34.5	84.4	36.4	26.2	1.94
285	20.0	447.3	389.0	36.5	82.2	35.3	25.3	2.18
300	20.0	447.3	389.0	35.5	86.7	35.5	26.0	2.18
330	-6.0	496.0	418.7	37.0	80.0	35.6	24.6	2.30
360	-17.0	779.4	631.2	40.2	77.4	33.2	22.2	2.50
390	-20.0	734.2	631.2	40.1	73.3	33.7	23.1	
420	-21.0	817.9	676.3	41.0	69.6	26.6	18.1	
480	-23.0	842.2	694.4	40.7	64.4	19.8	13.1	
540	-24.0	799.6	664.2	40.9	68.7	14.7		
570	-24.5	819.0	632.0	40.0	70.7	16.5	11.4	
585	-25.0	819.0	632.0	40.2	73.3	18.4	12.1	
600	-25.0	819.0	632.0	39.5	78.7	19.4	13.0	
630	10.0	499.5	339.4	38.0	75.3	11.7	7.4	
660	11.0	499.5	352.3	39.0	72.8	8.5	5.6	1.70

APPENDIX C - 5 GLUCOSE TRIAL 5 SHEEP # 1330

FED COLD WT. (KG)=68.0 INF. (UCX10-4/MIN)= 1554.4 1067.9

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG	C------	
240	20.0	458.2	360.1	32.0	72.6	19.6	14.1	1.87
270	20.0	458.2	386.3	33.4	70.7	20.3	15.2	2.06
285	20.0	517.3	419.1	34.2	70.7	20.6	14.7	2.11
300	20.0	484.7	399.4	32.5	79.0	20.2	14.7	1.99
330	-9.0	587.0	450.1	36.2	75.8	21.1	15.7	2.16
360	-18.0	626.2	495.7	37.0	82.5	20.0	14.1	2.23
390	-20.0	787.0	611.4	36.5	80.1	19.2	13.3	2.31
420	-20.0	630.8	500.8	36.2	84.2	17.3	12.2	2.48
480	-20.5	681.8	545.4	37.0	82.7	15.9	11.3	2.72
540	-21.0	733.5	577.6	37.2	78.5	14.2	10.2	2.83
570	-21.5	728.6	559.5	36.7	78.6	15.2	10.6	2.82
585	-22.0	728.6	579.0	36.7	73.9	15.6	11.1	2.67
600	-22.0	1001.7	774.1	0.0	77.7	15.6	10.9	2.74
630	10.0	468.4	364.3	34.5	71.4	18.0	12.4	2.50
660	14.0	449.0	351.3	33.7	74.0	18.5	13.1	2.42

APPENDIX C 6 GLUCOSE TRIAL 6 SHEEP # 1530

FED COLD WT. (KG)=66.5 INF. (UCX10-4/MIN)= 1758.1 1190.8

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	21.0	482.1	360.1	33.5	51.7	25.1	17.6	1.71
270	21.7	500.5	347.9	33.0	52.2	26.4	18.2	1.74
285	22.0	537.2	347.9	33.0	50.0	27.7	18.7	1.93
300	22.5	824.0	549.3	34.0	49.5	27.8	18.9	1.74
330	-6.0	620.3	411.5	32.9	50.3	25.0	17.0	2.35
360	-17.0	755.3	503.6	34.0	54.5	24.4	17.3	2.20
390	-21.0	838.5	561.1	35.0	56.0	23.9	16.2	2.34
420	-18.4	703.0	474.8	34.5	51.1	23.2	16.3	2.58
480	-18.0	847.1	564.7	34.5	53.2	19.7	13.0	2.87
540	-24.0	927.5	638.8	35.0	57.0	0.0	0.0	2.23
570	-25.0	916.7	635.6	35.0	59.0	0.0	0.0	2.21
585	-25.5	812.9	592.9	34.5	58.6	0.0	0.0	2.16
600	-26.0	1149.1	819.0	36.0	58.5	0.0	0.0	2.63
630	15.0	454.0	386.8	33.0	53.6	19.7	12.6	2.41
660	19.5	398.7	306.7	33.0	50.0	23.6	14.5	2.30

APPENDIX C 7 GLUCOSE TRIAL 7 SHEEP # 1080

FAST WARM WT. (KG)=80.0 INF. (UCX10-4/MIN)= 1445.8 1011.7

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	19.0	0.0	0.0	33.5	55.8	0.0	0.0	0.0
270	19.0	345.1	243.5	35.0	59.2	41.4	27.9	2.33
285	19.0	446.4	277.3	35.1	55.8	39.8	26.6	2.39
300	19.0	608.6	412.6	36.1	78.8	30.8	20.1	2.14
330	-9.0	379.4	270.9	33.5	68.9	36.6	24.5	2.10
360	-17.0	535.0	372.5	35.0	69.4	39.0	25.8	2.04
390	-18.5	601.0	418.7	34.5	68.4	36.9	23.5	2.02
420	-19.0	654.9	486.2	34.5	70.1	37.0	24.3	1.97
480	-21.0	795.6	586.5	35.2	72.7	34.0	21.3	2.25
540	-22.0	711.6	542.2	33.8	73.1	34.3	21.0	2.27
570	-22.5	940.0	669.6	35.0	75.5	33.8	21.5	2.78
585	-22.7	933.5	656.1	35.0	69.8	33.6	21.4	2.84
600	-23.0	906.2	676.4	35.8	69.3	35.1	21.4	2.89
630	12.0	413.5	311.8	32.9	68.6	36.8	22.8	2.64
660	16.0	386.5	298.3	31.5	55.8	37.9	23.8	2.55

APPENDIX C 8 GLUCOSE TRIAL 8 SHEEP # 1090

FAST WARM WT. (KG)=70.0 INF. (UCX10-4/MIN)= 1782.1 1225.3

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG		C------
240	21.5	261.4	194.5	33.0	77.0	0.0	0.0	2.48
270	22.0	249.2	182.4	31.5	73.6	0.0	0.0	2.64
285	22.0	255.4	182.4	32.8	74.2	55.8	39.4	2.96
300	22.0	534.9	358.7	39.0	70.3	53.8	37.7	2.69
330	-4.5	564.7	388.6	38.0	72.5	48.0	32.8	3.09
360	-14.0	576.7	412.8	36.5	62.4	46.0	31.3	3.54
390	-20.0	558.5	406.7	37.0	58.4	45.4	30.5	4.04
420	-21.5	564.5	424.9	38.5	58.6	49.2	33.5	4.27
480	-21.0	516.1	358.2	38.7	55.7	51.5	32.2	4.19
540	-20.5	543.0	398.3	37.8	50.3	45.7	29.2	4.07
570	-21.0	585.5	418.8	38.5	51.6	44.7	27.8	4.37
585	-21.0	627.2	448.7	38.5	50.4	42.5	27.3	4.29
600	-21.0	627.2	448.7	39.5	51.2	44.7	27.3	4.54
630	13.0	360.8	252.6	36.2	47.6	45.2	28.2	4.33
660	17.5	360.8	252.6	36.1	50.6	41.5	25.6	3.89

APPENDIX C 9 GLUCOSE TRIAL 9 SHEEP # 1020

FAST WARM WT. (KG)=63.0 INF. (UCX10-4/MIN)= 1426.2 979.6

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2 C------
						------UCX10-4/MG		
240	25.0	295.8	227.0	31.8	78.8	0.0	0.0	1.98
270	25.0	288.9	220.1	31.0	72.6	0.0	0.0	2.19
285	25.0	282.0	220.1	31.0	73.5	53.3	38.1	2.25
300	25.0	364.7	261.4	36.1	77.6	50.6	36.0	2.70
330	-2.5	499.9	362.9	36.0	77.3	43.5	30.7	2.47
360	-12.0	554.6	383.5	35.1	78.3	39.5	27.2	2.50
390	-16.5	566.4	409.1	35.6	83.3	37.9	25.1	2.49
420	-19.0	614.3	443.1	36.0	77.3	37.5	24.9	2.83
480	-20.0	567.7	378.4	36.7	77.6	31.9	20.1	3.83
540	-22.0	620.7	457.0	39.0	65.2	30.4	19.2	3.97
570	-23.5	668.2	490.9	39.8	60.6	29.5	18.6	3.95
585	-21.0	599.9	436.4	37.2	58.1	30.0	19.1	3.86
600	-20.0	579.5	402.3	36.4	52.6	34.4	21.5	3.59
630	13.0	391.5	274.8	34.0	52.4	32.5	21.0	3.50
660	16.5	336.5	247.3	35.0	55.2	30.3	18.4	3.40

APPENDIX C 10 GLUCOSE TRIAL 10 SHEEP # 1110

FAST COLD WT. (KG)=77.0 INF. (UCX10-4/MIN)= 1338.3 920.0

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	22.0	450.9	348.4	33.7	90.1	0.0	0.0	0.91
270	22.0	410.0	314.3	33.8	92.6	0.0	0.0	1.01
285	22.5	389.3	307.4	33.2	87.0	29.9	21.7	1.24
300	22.5	410.0	273.3	34.5	85.4	29.3	21.0	1.17
330	-1.0	863.6	616.9	40.0	84.3	33.3	24.1	1.14
360	-14.0	548.3	404.4	34.8	85.0	31.6	22.7	1.19
390	-20.0	809.3	603.5	37.0	84.1	32.7	23.2	1.33
420	-22.5	843.4	637.8	38.0	81.4	31.6	22.1	1.56
480	-22.0	758.1	553.2	37.5	86.7	23.1	16.0	1.73
540	-21.5	871.2	619.4	39.0	81.9	26.1	17.6	1.59
570	-22.0	822.4	543.7	38.0	85.1	26.7	17.9	1.57
585	-22.0	815.6	537.0	37.5	81.9	27.3	18.4	1.62
600	-22.0	856.2	550.6	39.0	82.8	28.3	19.3	1.67
630	15.0	532.0	327.4	35.1	78.5	29.1	19.4	1.85
660	19.0	518.4	307.0	35.0	81.4	30.1	20.1	1.97

APPENDIX C 11 GLUCOSE TRIAL 11 SHEEP # 1330

FAST COLD WT. (KG)=68.0 INF. (UCX10-4/MIN)= 1825.7 1255.2

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	20.0	350.1	264.1	27.2	68.7	29.3	20.4	1.78
270	20.0	344.0	258.0	31.2	69.1	32.4	22.5	2.02
285	20.0	337.8	245.7	27.3	73.8	31.7	22.2	2.45
300	20.0	700.3	491.4	33.3	75.4	32.8	22.5	2.16
330	-8.0	423.3	306.7	28.3	82.7	29.7	19.7	2.15
360	-17.0	423.3	306.7	29.3	83.5	29.3	19.7	2.43
390	-21.0	453.8	0.0	0.0	0.0	0.0	0.0	0.0
420	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
480	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
540	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
570	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
585	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
630	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
660	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

APPENDIX C 12 GLUCOSE TRIAL 12 SHEEP # 1530

FAST COLD WT. (KG)=70.5 INF. (UCX10-4/MIN)= 1857.8 1277.3

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	21.5	371.3	264.4	36.2	80.9	37.2	26.3	2.00
270	21.6	377.8	270.7	33.8	76.4	37.4	26.5	2.39
285	21.8	320.4	238.7	33.0	77.2	37.7	26.7	2.58
300	22.0	464.8	332.9	34.3	75.4	38.6	26.8	2.47
330	3.0	464.8	332.8	34.0	79.6	38.2	26.1	2.41
360	-6.0	458.5	345.4	34.3	80.2	37.3	25.6	2.38
390	-17.0	576.2	413.3	34.2	85.3	37.7	24.9	2.05
420	-22.0	707.6	519.8	36.0	87.0	37.3	26.0	2.08
480	-21.0	589.9	426.8	33.8	76.9	38.7	26.2	2.26
540	-22.0	720.6	507.5	36.7	70.1	38.3	25.9	2.48
570	-22.0	636.3	449.1	34.0	71.0	37.5	24.0	2.53
585	-22.3	667.5	486.5	34.0	72.8	36.5	23.8	2.51
600	-22.5	661.2	486.5	35.0	73.8	36.1	23.8	2.51
630	12.0	376.6	276.3	33.2	75.6	36.8	23.5	3.16
660	14.0	364.1	257.4	33.1	75.1	32.9	20.8	3.30

APPENDIX C 13 GLUCOSE TRIAL 13 SHEEP # 1110

FED WT. (KG)=77.0 INF. (UCX10-4/MIN)= 1428.4 982.2

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	-3.0	579.8	453.6	37.1	74.6	19.4	13.9	1.43
270	-2.0	550.9	423.4	37.5	81.1	17.2	10.1	1.53
285	-3.0	571.9	447.6	38.2	81.3	20.1	14.4	1.50
300	-4.0	540.4	429.4	38.0	81.9	19.5	14.5	1.57

APPENDIX C 14 GLUCOSE TRIAL 14 SHEEP # 1330

FED WT. (KG)=68.0 INF. (UCX10-4/MIN)= 1913.7 1315.9

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	-0.5	595.4	445.1	34.0	53.9	20.2	14.4	2.46
270	-0.5	517.3	378.9	30.1	55.4	21.0	15.5	2.44
285	-0.5	487.4	349.0	30.5	53.7	21.8	16.0	2.53
300	-0.5	487.4	349.0	37.0	52.6	0.0	0.0	2.53

APPENDIX C 15 GLUCOSE TRIAL 15 SHEEP # 1530

FED WT. (KG)=71.0 INF. (UCX10-4/MIN)= 1422.9 978.4

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	-4.0	647.1	445.3	33.7	89.1	19.7	14.0	2.43
270	-4.0	498.8	356.2	34.8	88.9	14.5	10.2	1.63
285	-4.0	437.6	354.8	34.8	89.7	12.6	9.2	1.40
300	-4.0	449.4	372.5	32.5	94.4	14.8	11.1	1.41

APPENDIX C 16 GLUCOSE TRIAL 16 SHEEP # 1110

FAST WT. (KG)=76.0 INF. (UCX10-4/MIN)= 1430.5 983.9

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG C------		
240	-2.0	714.6	460.4	32.7	77.1	26.4	18.9	1.23
270	-2.0	673.2	434.0	32.0	75.4	29.2	20.1	1.37
285	-2.0	846.7	539.8	37.0	79.1	28.9	20.3	1.51
300	-3.0	1154.8	770.2	43.0	88.8	26.0	18.6	1.73

APPENDIX C 17 GLUCOSE TRIAL 17 SHEEP # 1330

FAST WT. (KG)=68.0 INF. (UCX10-4/MIN)= 1429.5 982.9

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2
						------UCX10-4/MG		C------
240	-2.0	581.5	344.7	34.5	88.2	25.0	17.8	1.29
270	-2.5	600.0	356.5	31.0	84.4	27.3	18.9	1.40
285	-3.0	600.0	356.5	30.1	82.1	27.8	20.7	1.40
300	-3.0	1146.8	713.1	34.5	79.8	27.4	18.8	1.71

APPENDIX C 18 GLUCOSE TRIAL 18 SHEEP # 1530

FAST WT. (KG)=71.0 INF. (UCX10-4/MIN)= 1957.4 1319.7

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	GLUCOSE CONC. (MG%)	--SA GLUCOSE--- U-14C-GL	1-3H-GL	SA CO2 C------
						------UCX10-4/MG		
240	-4.0	527.3	373.3	31.5	83.1	27.6	19.1	1.75
270	-4.0	515.2	308.2	31.0	83.8	36.9	27.0	2.06
285	-4.0	556.3	355.5	31.3	83.2	41.0	30.2	2.56
300	-4.0	544.3	331.9	31.0	80.0	33.8	23.8	3.53

APPENDIX C 19 PALMITATE TRIAL 1 SHEEP # 1080

FED WARM WT. (KG)=86.0 INF. (UCX10-4/MIN)= 1164.2

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	10.0	392.6	294.5	28.0	0.94	46.3	2.59
270	10.0	412.2	268.3	27.0	0.0	0.0	2.39
285	10.0	417.8	267.6	0.0	0.0	0.0	2.62
300	10.0	417.8	267.6	31.2	1.20	43.0	2.62
330	-12.0	686.8	614.9	33.6	0.0	0.0	1.09
360	-16.0	686.8	621.3	0.0	0.0	0.0	1.08
390	-19.0	686.8	621.3	32.5	3.91	24.8	1.47
420	-20.0	686.8	608.2	31.6	7.08	16.5	3.31
480	-20.0	494.9	475.2	30.0	2.30	33.1	2.81
540	-22.0	518.6	453.7	29.9	2.61	31.1	2.93
570	0.0	0.0	0.0	0.0	0.0	0.0	0.0
585	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	-23.5	579.2	482.7	29.6	4.00	24.4	2.80
630	10.0	285.7	253.1	28.5	1.71	37.7	2.63
660	16.0	356.9	279.1	29.5	1.90	36.1	3.04

APPENDIX C 20 PALMITATE TRIAL 2 SHEEP # 1090

FED WARM WT. (KG)=72.0 INF. (UCX10-4/MIN)= 1328.7

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	20.0	385.1	312.4	28.8	0.58	70.7	1.29
270	20.0	345.9	285.8	30.0	1.00	62.1	1.74
285	20.0	341.0	281.2	28.9	1.82	50.2	1.69
300	20.0	321.3	274.5	28.0	1.97	48.5	2.16
330	-8.0	789.9	568.3	31.0	4.43	31.2	3.18
360	-17.0	910.1	675.3	32.0	4.75	29.8	2.22
390	-18.0	571.5	436.2	29.5	2.25	45.6	2.01
420	-19.5	691.7	536.9	31.5	2.42	44.0	2.45
480	-20.5	659.8	492.6	33.4	2.46	43.7	2.52
540	-22.0	652.5	478.8	35.0	2.72	41.4	2.98
570	-23.0	711.4	511.8	0.0	0.0	0.0	2.80
585	-23.0	696.6	492.6	0.0	0.0	0.0	2.79
600	-23.0	755.5	531.0	34.8	4.06	32.9	2.92
630	10.0	444.0	321.2	33.8	3.14	38.4	3.08
660	13.0	436.6	314.8	33.7	4.33	31.6	3.24

APPENDIX C 21 PALMITATE TRIAL 3 SHEEP # 1020

FED WARM WT. (KG)=65.0 INF. (UCX10-4/MIN)= 1144.3

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	20.5	334.4	264.2	31.2	0.91	60.7	1.26
270	20.5	323.3	270.0	32.0	1.08	57.9	1.18
285	20.5	316.7	258.3	32.0	0.67	65.4	1.06
300	20.5	305.6	264.2	32.0	0.78	63.2	1.08
330	-4.0	516.0	416.8	36.0	1.96	46.3	1.77
360	-14.0	770.6	613.5	37.2	2.42	42.0	1.96
390	-19.5	653.3	513.9	36.6	2.53	41.1	2.08
420	-22.0	713.1	554.8	0.0	2.07	45.2	2.21
480	-22.0	617.8	465.9	34.4	3.51	34.3	2.16
540	-21.0	642.2	478.0	34.0	5.61	25.4	2.22
570	-21.0	721.9	523.5	35.0	8.39	18.9	2.33
585	-21.0	721.9	523.5	35.0	5.76	24.9	2.33
600	-21.0	733.0	529.3	35.5	8.42	18.8	2.31
630	13.0	314.5	238.1	30.0	2.53	41.0	4.28
660	14.0	365.4	278.7	32.0	4.18	30.9	2.24

APPENDIX C 22 PALMITATE TRIAL 4 SHEEP # 1110

FED COLD WT. (KG)=79.0 INF. (UCX10-4/MIN)= 1192.6

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (MI/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	20.0	613.7	468.1	32.0	1.63	42.9	1.21
270	20.0	492.5	404.5	32.0	0.78	54.2	1.35
285	20.0	438.7	352.5	32.0	2.17	37.9	1.38
300	20.0	457.5	369.9	32.0	1.27	47.1	1.49
330	-4.0	686.3	508.2	36.2	0.84	53.3	1.61
360	-16.0	842.4	612.1	37.5	1.83	41.0	1.85
390	-18.5	788.6	628.0	36.5	3.80	28.1	1.88
420	-22.0	721.3	524.3	36.5	0.78	54.2	1.77
480	-22.0	904.3	663.9	37.2	1.33	46.3	1.81
540	-23.0	721.3	540.5	38.0	1.91	40.2	1.80
570	-23.0	737.5	529.2	37.0	2.83	33.2	1.82
585	-24.0	715.9	540.5	37.0	2.05	38.9	1.80
600	-24.0	702.5	529.2	0.0	2.10	38.6	1.94
630	10.0	514.1	398.8	35.0	1.19	48.1	1.89
660	13.0	659.4	485.5	33.5	5.27	22.7	2.06

APPENDIX C 23 PALMITATE TRIAL 5 SHEEP # 1330

FED COLD WT. (KG)=70.0 INF. (UCX10-4/MIN)= 1218.1

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	18.0	448.8	349.7	30.0	0.76	62.9	1.41
270	18.0	435.5	329.9	27.5	0.0	0.0	1.46
285	18.0	729.3	532.2	33.0	1.02	58.3	2.07
300	18.0	755.8	565.1	34.0	1.96	45.8	2.39
330	-9.0	512.5	400.8	31.2	2.13	44.1	2.07
360	-19.0	577.4	446.2	33.3	2.06	44.8	2.40
390	-20.0	735.0	525.0	0.0	7.46	20.4	3.21
420	-21.0	741.4	531.6	34.0	2.35	42.1	2.88
480	-22.0	648.7	465.3	34.0	5.66	24.9	2.69
540	-23.5	793.7	539.9	35.5	5.64	25.0	2.81
570	-24.0	754.6	507.4	0.0	0.0	0.0	2.51
585	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	-25.0	709.0	481.4	34.9	5.16	26.6	2.81
630	10.0	424.3	280.6	32.0	3.55	33.7	2.71
660	13.0	372.0	267.6	30.5	1.36	53.1	2.96

APPENDIX C 24 PALMITATE TRIAL 6 SHEEP # 1530

FED COLD WT. (KG)=80.0 INF. (UCX10-4/MIN)= 1263.1

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	13.0	0.0	0.0	31.0	0.53	61.5	0.0
270	13.0	727.4	534.7	30.5	1.55	45.9	1.41
285	13.0	432.3	319.4	29.0	0.71	57.9	1.78
300	13.0	384.6	285.2	30.3	1.78	43.4	2.23
330	-10.0	527.1	427.9	31.5	1.27	49.3	2.57
360	-16.0	651.1	483.7	30.5	1.10	51.6	2.87
390	-20.0	502.3	384.4	30.1	1.10	51.5	2.63
420	-21.0	750.6	589.3	33.5	1.66	44.6	3.19
480	-23.0	726.6	527.0	32.6	3.29	32.0	2.96
540	-24.0	681.9	477.4	32.5	3.17	32.6	2.48
570	-24.5	798.6	552.9	0.0	0.0	0.0	2.80
585	-25.0	798.6	552.9	0.0	0.0	0.0	2.70
600	-25.0	737.3	509.9	32.5	4.13	27.9	2.70
630	7.0	387.0	279.0	31.0	2.51	37.0	2.55
660	12.0	399.3	291.4	31.5	2.50	37.0	3.04

APPENDIX C 25 PALMITATE TRIAL 7 SHEEP # 1080

FAST WARM WT. (KG)=79.0 INF. (UCX10-4/MIN)= 1273.9

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	19.0	306.8	205.3	32.0	2.51	37.7	2.96
270	19.0	314.9	212.1	32.0	2.35	39.0	3.31
285	19.0	314.9	219.9	31.6	3.39	32.1	3.12
300	19.0	314.9	219.6	31.8	4.61	26.6	3.10
330	-8.0	705.2	492.1	34.4	12.04	12.9	3.43
360	-18.0	710.5	478.5	32.6	6.58	20.8	3.07
390	-22.0	667.5	465.6	33.0	6.07	22.0	3.38
420	-22.0	654.0	452.1	32.5	5.66	23.1	3.15
480	-24.0	710.5	473.2	31.5	6.77	20.3	2.79
540	-27.0	807.4	527.3	30.8	9.11	16.2	2.95
570	-27.0	866.6	554.0	0.0	0.0	0.0	2.75
585	-27.0	815.5	534.0	0.0	0.0	0.0	2.69
600	-27.0	861.3	500.6	31.2	8.32	17.4	3.16
630	8.5	368.7	248.4	29.0	4.81	25.8	2.95
660	12.0	328.4	235.0	28.0	3.54	31.3	3.87

APPENDIX C 26 PALMITATE TRIAL 8 SHEEP # 1090

FAST WARM WT. (KG)=69.0 INF. (UCX10-4/MIN)= 1145.7

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	23.0	242.1	180.7	29.0	2.14	42.0	2.93
270	23.0	216.3	165.2	29.0	2.33	40.3	3.50
285	23.0	228.0	165.2	28.6	3.14	34.5	3.94
300	23.0	237.4	175.5	29.0	3.37	33.2	4.12
330	-4.0	580.6	388.2	32.7	10.08	15.4	4.04
360	-14.0	672.3	465.9	32.3	6.71	21.1	3.58
390	-21.0	754.6	525.8	33.0	4.63	27.2	3.46
420	-23.0	738.1	520.6	32.0	4.76	26.8	3.32
480	-21.0	705.2	495.8	32.0	3.98	30.0	3.34
540	-21.0	756.9	535.7	33.0	4.07	29.6	3.34
570	-21.0	712.3	491.8	33.0	5.90	23.1	3.08
585	-21.0	733.4	502.2	33.0	6.29	22.1	3.01
600	-21.0	721.7	491.8	33.0	7.19	20.0	2.98
630	11.0	453.7	312.9	33.0	3.42	32.9	3.11
660	16.0	437.2	307.7	32.0	6.46	21.7	3.35

APPENDIX C 27 PALMITATE TRIAL 9 SHEEP # 1020

FAST WARM WT. (KG)=70.5 INF. (UCX10-4/MIN)= 1178.3

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	18.0	521.2	452.7	32.8	2.20	41.8	3.20
270	18.0	324.3	243.2	32.8	2.39	40.1	3.76
285	18.0	372.3	263.3	32.8	3.15	34.7	3.95
300	18.0	357.9	269.7	32.9	2.66	38.0	4.22
330	-10.0	742.2	512.5	35.0	0.0	0.0	2.82
360	-20.0	708.5	512.5	35.3	5.62	24.1	3.44
390	-21.0	718.2	523.1	35.0	5.02	26.0	3.83
420	-22.0	725.4	523.1	33.9	5.34	24.9	2.39
480	-24.0	840.7	571.8	34.0	5.30	25.1	2.68
540	-25.0	823.8	563.4	34.0	8.50	17.8	2.33
570	-26.0	771.0	522.8	0.0	0.0	0.0	2.39
585	-26.5	771.0	522.8	0.0	0.0	0.0	2.39
600	-27.0	790.2	529.7	34.0	11.32	14.1	2.42
630	8.0	338.7	222.9	30.5	5.79	23.6	2.55
660	11.0	372.3	243.2	30.0	4.60	27.5	2.85

APPENDIX C 28 PALMITATE TRIAL 10 SHEEP # 1110

FAST COLD WT. (KG)=76.5 INF. (UCX10-4/MIN)= 1188.8

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	23.0	474.3	340.2	40.0	4.70	25.3	2.17
270	23.0	534.3	376.7	42.0	4.72	25.2	2.33
285	23.0	492.6	334.1	40.0	5.69	22.2	2.55
300	23.0	474.3	328.1	40.5	5.74	22.0	2.56
330	-8.0	448.3	305.3	40.8	4.12	27.5	2.29
360	-17.0	823.6	567.0	43.0	8.06	17.2	2.56
390	-21.0	682.8	472.2	43.0	7.54	18.1	2.64
420	-20.0	750.6	534.4	43.0	3.63	29.7	2.50
480	-21.0	862.7	574.7	43.0	4.29	26.8	2.48
540	-22.0	867.9	585.8	43.0	5.26	23.4	2.57
570	-23.0	774.1	528.0	43.0	4.42	26.3	2.33
585	-23.0	774.1	528.0	42.6	4.60	25.6	2.33
600	-23.0	844.4	568.2	44.0	5.63	22.3	2.40
630	15.0	443.1	314.5	41.0	3.99	28.1	2.42
660	17.0	414.4	289.9	41.1	2.81	34.3	2.70

APPENDIX C 29 PALMITATE TRIAL 12 SHEEP # 1530

FAST COLD WT. (KG)=66.0 INF. (UCX10-4/MIN)= 1196.2

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	23.0	463.2	333.4	41.0	6.03	24.9	2.41
270	23.0	422.7	298.6	41.0	3.82	33.6	2.72
285	23.0	368.8	293.6	40.5	4.09	32.2	2.85
300	23.0	348.5	278.7	41.0	5.53	26.4	2.92
360	-7.0	598.1	437.8	43.0	3.59	34.9	2.85
360	-15.0	670.1	473.0	42.0	5.12	27.8	2.83
390	-21.5	793.7	547.4	44.0	7.40	21.4	2.88
420	-22.0	593.6	426.8	42.5	6.96	22.4	2.48
480	-22.0	757.8	527.5	44.0	4.40	30.8	2.80
540	-23.0	652.1	446.1	42.5	4.25	31.4	2.81
570	-23.5	780.2	558.6	43.5	4.17	31.8	2.84
585	-23.7	775.8	538.5	43.0	4.66	29.6	2.81
600	-24.0	744.3	513.4	43.5	0.0	0.0	2.82
630	9.0	404.7	262.2	41.0	4.01	32.6	2.78
660	14.0	398.0	252.1	41.0	4.59	29.9	2.99

APPENDIX C 30 PALMITATE TRIAL 13 SHEEP # 1110

FED WT. (KG)=79.0 INF. (UCX10-4/MIN)= 1061.5

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	0.0	516.8	373.5	35.5	0.0	0.0	1.12
270	0.0	506.0	373.5	35.5	0.0	0.0	1.35
285	1.0	487.2	349.7	34.0	2.43	32.0	1.50
300	2.0	503.3	376.2	34.0	2.87	29.3	1.58

APPENDIX C 31 PALMITATE TRIAL 15 SHEEP # 1530

FED WT. (KG)=66.0 INF. (UCX10-4/MIN)= 1173.7

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (MI/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	-3.0	577.9	419.8	33.0	2.64	40.6	1.32
270	-3.0	553.1	409.9	32.0	2.06	45.7	1.57
285	-3.0	535.2	388.2	31.0	3.15	36.9	1.67
300	-3.0	530.7	383.3	0.0	2.98	38.0	1.73

APPENDIX C 32 PALMITATE TRIAL 16 SHEEP # 1110

FAST WT. (KG)=79.0 INF. (UCX10-4/MIN)= 1228.8

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	1.0	538.3	336.6	32.5	0.0	0.0	1.53
270	0.0	616.3	434.9	32.5	3.61	29.8	2.56
285	0.0	427.9	289.6	31.0	2.68	35.2	2.33
300	0.0	433.3	289.6	30.2	1.81	42.4	2.42

APPENDIX C 33 PALMITATE TRIAL 18 SHEEP # 1530

FAST WT. (KG)=66.0 INF. (UCX10-4/MIN)= 1148.8

TIME (MIN.)	TEMP. (C)	O2 CONSUMP. (ML/MIN)	CO2 PROD. (ML/MIN)	HEM-ATOCRIT (%)	PALM CN. (UMOLE/ ML)	SA PALM. (UCX10-4 /MG C)	SA CO2 (UCX10-4 /MG C)
240	-4.0	461.0	317.6	27.7	4.47	29.2	2.25
270	-4.0	571.1	387.0	0.0	9.57	16.8	2.62
285	-4.0	544.1	351.0	0.0	5.53	25.3	2.68
300	-4.0	519.4	355.9	27.5	5.17	26.6	2.59

APPENDIX D

Composition of Plasma Free Fatty Acids

The composition of the plasma FFA's from the palmitate trials is shown below along with the heat of combustion of each of the FFA's measured. These values have been multiplied together to give the relative amount of energy in each FFA fraction.

FFA	Mean Value (range) (umole/100 umole)		Heat of Combustion (cal/umole FFA)	(cal/100 umole)
16:0	17.8	(13.3-21.6)	2.398	42.69
16:1	2.4	(1.1- 5.5)	2.355	5.65
17:0	2.4	(1.1- 5.0)	2.554	6.13
17:1	1.3	(0.1- 4.0)	2.510	3.26
18:0	25.7	(11.6-35.4)	2.712	69.69
18:1	39.5	(31.5-49.0)	2.666	105.30
18:2	7.5	(3.7-16.1)	2.623	19.67
18:3	3.1	(1.4- 5.2)	2.579	8.00
				260.39

The percent of the plasma FFA energy contained in the palmitate fraction =

$$\frac{42.69}{260.39} \times 100 = *16.3\%$$

* This value should probably be slightly lower since it does not account for the FFA's with chain lengths less than 16:0.